National Cancer Institute
CARCINOGENESIS
Technical Report Series
No. 114
1978

BIOASSAY OF 2,3,5,6-TETRACHLORO-4-NITROANISOLE FOR POSSIBLE CARCINOGENICITY

CAS No. 2438-88-2

NCI-CG-TR-114

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
National Institutes of Health

BIOASSAY OF

2,3,5,6-TETRACHLORO-4-NITROANISOLE

FOR POSSIBLE CARCINOGENICITY

Carcinogenesis Testing Program
Division of Cancer Cause and Prevention
National Cancer Institute
National Institutes of Health
Bethesda, Maryland 20014

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
National Institutes of Health

DHEW Publication No. (NIH) 78-1369

REPORT ON THE BIOASSAY OF 2,3,5,6-TETRACHLORO-4-NITROANISOLE FOR POSSIBLE CARCINOGENICITY

CARCINOGENESIS TESTING PROGRAM
DIVISION OF CANCER CAUSE AND PREVENTION
NATIONAL CANCER INSTITUTE, NATIONAL INSTITUTES OF HEALTH

FOREWORD: This report presents the results of the bioassay of 2,3,5,6-tetrachloro-4-nitroanisole conducted for the Carcinogenesis Testing Program, Division of Cancer Cause and Prevention, National Cancer Institute (NCI), National Institutes of Health, Bethesda, Maryland. This is one of a series of experiments designed to determine whether selected chemicals have the capacity to produce cancer in animals. Negative results, in which the test animals do not have a significantly greater incidence of cancer than control animals, do not necessarily mean the test chemical is not a carcinogen because the experiments are conducted under a limited set of circumstances. Positive results demonstrate that the test chemical is carcinogenic for animals under the conditions of the test and indicate a potential risk to man. The actual determination of the risk to man from animal carcinogens requires a wider analysis.

CONTRIBUTORS: This bioassay of 2,3,5,6-tetrachloro-4-nitroanisole was conducted by Mason Research Institute, Worcester, Massachusetts, initially under direct contract to the NCI and currently under a subcontract to Tracor Jitco, Inc., prime contractor for the NCI Carcinogenesis Testing Program.

The experimental design was determined by the NCI Project Officers, Dr. J. H. Weisburger (1,2) and Dr. E. K. Weisburger (1). The principal investigators for the contract were Dr. E. Smith (3) and Dr. A. Handler (3). Animal treatment and observation were supervised by Mr. G. Wade (3) and Ms. E. Zepp (3). Chemical analysis was performed by Mason Research Institute (3) and Midwest Research Institute (4) and the analytical results were reviewed by Dr. N. Zimmerman (5).

Histopathologic examinations were performed by Dr. R. L. Schueler (6) as a consultant for Mason Research Institute, and the diagnoses included in this report represent the interpretation of this pathologist.

Compilation of individual animal survival, pathology, and summary tables was performed by EG&G Mason Research Institute (7); the statistical analysis was performed by Mr. W. W. Belew (5,8), using methods selected for the Carcinogenesis Testing Program by Dr. J. J. Gart (9).

This report was prepared at METREK, a Division of The MITRE Corporation (5) under the direction of the NCI. Those responsible for this report at METREK are the project coordinator, Dr. L. W. Thomas (5), task leader Dr. M. R. Kornreich (5,10), senior biologist Ms. P. Walker (5), biochemist Mr. S. C. Drill (5), and technical editor Ms. P. A. Miller (5). The final report was reviewed by members of the participating organizations.

The following other scientists at the National Cancer Institute were responsible for evaluating the bioassay experiment, interpreting the results, and reporting the findings: Dr. K. C. Chu (1), Dr. C. Cueto, Jr. (1), Dr. J. F. Douglas (1), Dr. D. G. Goodman (1,10), Dr. R. A. Griesemer (1), Dr. M. H. Levitt (1), Dr. H. A. Milman (1), Dr. T. W. Orme (1), Dr. R. A. Squire (1,11), Dr. S. F. Stinson (1), Dr. J. M. Ward (1), and Dr. C. E. Whitmire (1).

---

1. Carcinogenesis Testing Program, Division of Cancer Cause and Prevention, National Cancer Institute, National Institutes of Health, Bethesda, Maryland.

2. Now with the Naylor Dana Institute for Disease Prevention, American Health Foundation, Hammon House Road, Valhalla, New York.

3. Mason Research Institute, 57 Union Street, Worcester, Massachusetts.

4. Midwest Research Institute, 425 Volker Boulevard, Kansas City, Missouri.

5. The MITRE Corporation, METREK Division, 1820 Dolley Madison Boulevard, McLean, Virginia.

6. Tracor Jitco, Inc., 1776 East Jefferson Street, Rockville, Maryland.

7. EG&G Mason Research Institute, 1530 East Jefferson Street, Rockville, Maryland.

8. Now with the Solar Energy Research Institute, Cole Boulevard, Golden, Colorado.

9. Mathematical Statistics and Applied Mathematics Section, Biometry Branch, Field Studies and Statistics Program, Division of Cancer Cause and Prevention, National Cancer Institute, National Institutes of Health, Bethesda, Maryland.

10. Now with Clement Associates, Inc., 1010 Wisconsin Avenue, N.W., Washington, D.C.

11. Now with the Division of Comparative Medicine, Johns Hopkins University, School of Medicine, Traylor Building, Baltimore, Maryland.

SUMMARY

A bioassay for possible carcinogenicity of 2,3,5,6-tetrachloro-4-nitroanisole was conducted using Fischer 344 rats and B6C3F1 mice. 2,3,5,6-Tetrachloro-4-nitroanisole was administered in the feed, at either of two concentrations, to groups of male and female animals of each species. The high and low dietary concentrations used in the chronic bioassay were 0.012 and 0.006 percent, respectively, for both species. After a 104-week period of chemical administration, observation of rats continued for up to 3 weeks and observation of mice continued for up to 1 week. For rats 50 animals of each sex were placed on test as controls, while for mice 55 animals of each sex were placed on test as controls.

There were no significant positive associations between the dietary concentration of 2,3,5,6-tetrachloro-4-nitroanisole administered and mortality in rats or mice of either sex. Adequate numbers of animals in all groups survived sufficiently long to be at risk from late-developing tumors.

No neoplasms, except for interstitial-cell testicular tumors in males, occurred at statistically significant incidences in dosed rats. Because of the high and variable spontaneous incidence of these lesions in Fischer 344 rats, these tumors were not considered to be associated with the administration of the test compound.

Among dosed male mice the combined incidence of leukemia and malignant lymphoma was statistically significant. However, since these lesions occur spontaneously and with high variation in B6C3F1 mice, the lesions were not considered to be associated with the administration of the test compound. No neoplasms were of a statistically significant incidence in dosed female mice.

Under the conditions of this bioassay, dietary administration of 2,3,5,6-tetrachloro-4-nitroanisole was not carcinogenic to male or female Fischer 344 rats or B6C3F1 mice of either sex.

TABLE OF CONTENTS

TABLE OF CONTENTS (Concluded)

LIST OF ILLUSTRATIONS

LIST OF TABLES

LIST OF TABLES (Concluded)

I. INTRODUCTION

2,3,5,6-Tetrachloro-4-nitroanisole (Figure 1) (NCI No. C03032), an agricultural fungicide and acaricide, was selected for bioassay by the National Cancer Institute because of its structural similarity to pentachloronitrobenzene, a pesticide classified as tumorigenic by the Secretary's Commission on Pesticides and their Relationship to Environmental Health (U.S. Department of Health, Education, and Welfare, 1969).

The Chemical Abstracts Service (CAS) Ninth Collective Index (1977) name for this compound is 1-methoxy-4-nitro-2,3,5,6-tetrachlorobenzene.* It is also known as tetrachloronitroanisole; ENT 22335; and TCNA.

2,3,5,6-Tetrachloro-4-nitroanisole has been shown to control fungi which cause flag smut of winter wheat (Purdy, 1963) and rust, root and stem rot, and wilt in a variety of vegetables and grains (Carey, 1963). The compound's singular effectiveness against flag smut is a result of its ability to control infections arising from both seed-borne and soil-borne flag smut spores (Purdy, 1963). Nonetheless, 2,3,5,6-tetrachloro-4-nitroanisole is not currently registered as a pesticide in the United States (Schaughnessy, 1977).

Although specific production figures for 2,3,5,6-tetrachloro-4-nitroanisole are not available, its exclusion from Synthetic Organic

*The CAS registry number is 2438-88-2.

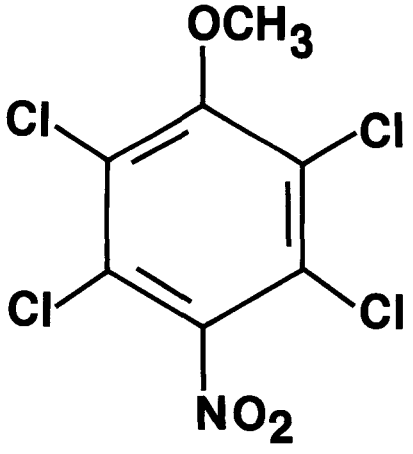


FIGURE 1
CHEMICAL STRUCTURE OF 2,3,5,6-TETRACHLORO-4-NITROANISOLE

Chemicals: United States Production and Sales, 1975 (U.S. International Trade Commission, 1977) implies that it is not produced in commercial quantities in the United States.

Since 2,3,5,6-tetrachloro-4-nitroanisole is not in present use as a pesticide, the potential for exposure is greatest for those persons engaged in the synthesis of 2,3,5,6-tetrachloro-4-nitroanisole or in agricultural research involving 2,3,5,6-tetrachloro-4-nitroanisole.

II. MATERIALS AND METHODS

A. Chemicals

2,3,5,6-Tetrachloro-4-nitroanisole was purchased from Carroll Products, Wood River Junction, Rhode Island. Chemical analysis was performed by Mason Research Institute, Worcester, Massachusetts and Midwest Research Institute, Kansas City, Missouri. The experimentally determined melting point range was 101° to 105°C. Two differing literature values (i.e., 105° to 106°C [Berckmans and Halleman, 1925] and 112° to 113°C [Peters et al., 1943]) were found with no adequate explanation for the variation. Elemental analysis of the purchased compound suggested the presence of at least minor impurities. Thin-layer chromatography utilizing two solvent systems (i.e., ethyl acetate:hexane and benzene:chloroform) revealed, respectively, two spots and one spot. Vapor-phase chromatography indicated two impurities of motility similar to the major compound. Nuclear magnetic resonance and infrared analyses were consistent with the structure of the compound.

Throughout this report, the term 2,3,5,6-tetrachloro-4-nitroanisole is used to refer to this compound.

B. Dietary Preparation

The basal laboratory diet for both dosed and control animals consisted of Wayne Lab-Blox® (Allied Mills, Inc., Chicago, Illinois). 2,3,5,6-Tetrachloro-4-nitroanisole was administered to the dosed animals as a component of the diet.

The chemical was removed from its container and proper amounts were sifted and weighed out under an exhaust hood. The compound was blended in an aluminum bowl with an aliquot of the ground feed. Once visual homogeneity was attained, the mixture was placed in a 6 kg capacity Patterson-Kelley twin-shell stainless steel V-blender along with the remainder of the meal. The blender was sealed and operated for 20 minutes. The mixtures were placed in double plastic bags and stored in the dark at 4°C. The mixture was prepared once weekly and unused portions were discarded 14 days after formulation.

C. Animals

Two animal species, rats and mice, were used in the carcinogenicity bioassay. Fischer 344 rats and B6C3F1 mice were obtained through contracts of the Division of Cancer Treatment, National Cancer Institute. All rats were supplied by Charles River Breeding Laboratories, Wilmington, Massachusetts, and all mice were supplied by the Frederick Cancer Research Center, Frederick, Maryland. Dosed and control animals for both species were received in separate shipments.

Upon arrival, a sample of animals was examined for parasites and other signs of disease. One group of high dose male rats (group 2 as defined in Section II. F., Experimental Design) had parasite infestations and were treated with 3.0 gm piperazine adipate per liter in drinking water for 3 days, followed by 3 days of tap water and 3 more days of piperazine adipate administration. Animals to be used in the chronic bioassay were quarantined by species for 2 weeks prior to

initiation of test. Animals were assigned to groups and distributed among cages so that average body weight per cage was approximately equal for a given sex and species.

D. Animal Maintenance

All animals were housed by species in rooms having a temperature range of 23° to 34°C. Incoming air was filtered through Tri-Dek® 15/40 denier Dacron® filters (Tri-Dim Filter Corp., Hawthorne, New Jersey) providing six changes of room air per hour. Fluorescent lighting was provided on a 12-hour-daily cycle.

Rats were housed five per cage by sex. During quarantine and for the first 12 months of study, dosed rats were held in galvanized- or stainless-steel wire-mesh cages (Fenco Cage Products, Boston, Massachusetts) suspended over newspapers (except for the high dose group 2 males, which were housed in wire-mesh cages for the first 10 months). Control rats were housed in wire-mesh cages for the first 14 months of study. Newspapers under cages were replaced daily, and cages and racks washed weekly. For the remainder of the study, all rats were housed in suspended polycarbonate cages (Lab Products, Inc., Garfield, New Jersey) equipped with disposable nonwoven fiber filter sheets. Clean bedding and cages were provided twice weekly. SAN-I-CEL® corncob bedding (Paxton Processing Company, Paxton, Illinois) was used for the first 6 months of polycarbonate caging. Aspen hardwood chip bedding (American Excelsior Company, Baltimore, Maryland) was then used for the remainder of the study. Stainless steel cage racks were

cleaned once every 2 weeks, and disposable filters were replaced at that time.

Mice were housed five per cage by sex in polycarbonate shoe box type cages. Cages were fitted with perforated stainless steel lids (Lab Products, Inc.). Nonwoven fiber filter bonnets were used over cage lids. Clean cages, lids, and bedding were provided twice per week. Bed-o-cobs® corncob bedding (The Andersons Cob Division, Maumee, Ohio) was used for the first 7 months of study for dosed mice, and for the first 8 months of study for control mice. Aspen bedding was used thereafter. Reusable filter bonnets and pipe racks were sanitized every 2 weeks throughout the study.

Water was available ad libitum from 250 ml water bottles equipped with rubber stoppers and stainless steel sipper tubes. Bottles were replaced twice weekly and, for rats only, water was supplied as needed between changes.

During the period of compound administration dosed animals were fed Wayne Lab-Blox® meal containing the appropriate concentration of 2,3,5,6-tetrachloro-4-nitroanisole. Control animals received untreated meal. Food was supplied to rats in Alpine® aluminum feed cups (Curtin Matheson Scientific, Inc., Woburn, Massachusetts) while they were in wire-mesh cages. While in polycarbonate cages, food was supplied to rats from stainless steel gangstyle feed hoppers (Scientific Cages, Inc., Bryan, Texas). Food was supplied to dosed and control mice from Alpine® feed cups for the first 1 and 2 months of

study, respectively, and from gangstyle hoppers thereafter. Food hoppers were changed on the same schedule as were cages. Food was replenished daily in Alpine® feed cups.

All dosed and control rats were housed in a room with other rats receiving diets containing* 4-chloro-o-phenylenediamine (95-83-0); acetylaminofluorene (53-96-3); p-cresidine (120-71-8); 4-chloro-m-phenylenediamine (5131-60-2); and 1H-benzotriazole (95-14-7).

Dosed mice were housed in a room with other mice receiving diets containing hydrazobenzene (530-50-7); tris(2,3-dibromopropyl) phosphate (126-72-7); N-(1-naphthyl)ethylenediamine dihydrochloride (1465-25-4); 2-chloro-p-phenylenediamine sulfate (61702-44-1); and aniline hydrochloride (142-04-1). Control mice were housed in a room with other mice receiving diets containing fenaminosulf (140-56-7); 2,5-dithiobiurea (142-46-1); 4-chloro-o-phenylenediamine (95-83-0); o-anisidine hydrochloride (134-29-0); p-anisidine hydrochloride (20265-97-8); and cupferron (135-20-6).

E. Selection of Initial Concentrations

In order to establish the maximum tolerated concentrations of 2,3,5,6-tetrachloro-4-nitroanisole for administration to dosed animals in the chronic studies, subchronic toxicity tests were conducted with both rats and mice. Rats were distributed among seven groups, each consisting of five males and five females. 2,3,5,6-Tetrachloro-4-nitroanisole was incorporated into the basal laboratory diet and

*CAS registry numbers are given in parentheses.

supplied ad libitum to seven of the eight rat groups in concentrations of 0.003, 0.01, 0.025, 0.05, 0.1, 0.2, and 0.4 percent. Mice were distributed among nine groups, each consisting of five males and five females. The chemical was incorporated into the laboratory diet and supplied ad libitum to eight of the nine mouse groups in concentrations of 0.003, 0.006, 0.0125, 0.025, 0.05, 0.1, 0.2, and 0.4 percent. One rat group and one mouse group each served as a control group, receiving only the basal laboratory diet. The dosed dietary preparations were administered for 8 weeks. Individual body weights were recorded during the first, fourth, and seventh weeks of the subchronic study. Survivors were sacrificed at the end of the test, and gross necropsies were performed.

The highest concentration causing no deaths, no compound-related gross abnormalities, and no mean body weight depression in excess of 10 percent relative to controls during the 8-week subchronic test was selected as the high concentration utilized for the chronic bioassay.

All rats receiving concentrations of 0.025 percent or more died. Each male rat receiving a concentration of 0.025 percent or higher had a spotted or colored liver or thymus. No gross pathology was observed in female rats. A dietary concentration of 0.01 percent produced a mean body weight depression of 8.2 percent in male rats and no mean weight depression in female rats. The high concentration selected for administration to rats in the chronic study was 0.012 percent.

All mice receiving concentrations of 0.1 percent or higher died. Two male and three female mice receiving 0.05 percent died, and one male mouse receiving 0.025 percent died. Mesenteric lymph nodes were moderately enlarged in female mice receiving 0.025 percent. A dietary concentration of 0.0125 produced mean body weight depressions of 10.8 and 6.3 percent in male and female mice, respectively. A dietary concentration of 0.006 percent produced mean body weight depressions of 10.0 and 7.2 percent in male and female mice, respectively. The high concentration selected for administration to mice in the chronic study was 0.012 percent.

F. Experimental Design

The experimental design parameters for the chronic bioassay (species, sex, group size, concentrations administered, and duration of treated and untreated observation periods) are summarized in Tables 1 and 2.

All rats were approximately 6 weeks old at the time they were placed on test. The concentrations of 2,3,5,6-tetrachloro-4-nitroanisole in diets were 0.012 and 0.006 percent. Throughout this report those rats receiving the former concentration are referred to as the high dose groups and those receiving the latter concentration are referred to as the low dose groups. The high dose male rat group was improperly sexed, and all females that were included in the male group were removed from the study. Therefore, approximately 6 weeks after the start of the bioassay, a supplementary group of 25 male rats was

TABLE 1

DESIGN SUMMARY FOR FISCHER 344 RATS
2,3,5,6-TETRACHLORO-4-NITROANISOLE FEEDING EXPERIMENT

	INITIAL GROUP SIZE	2,3,5,6-TETRACHLORO-4-NITROANISOLE CONCENTRATION (PERCENT)	OBSERVATION PERIOD TREATED (WEEKS)	OBSERVATION PERIOD UNTREATED (WEEKS)
MALE				
CONTROL	50	0	0	105
LOW DOSE	49	0.006 0	104	1
HIGH DOSE 1	24	0.012 0	104	1
HIGH DOSE 2*	25	0.012 0	104	3
FEMALE				
CONTROL	50	0	0	105
LOW DOSE	50	0.006 0	104	1
HIGH DOSE	50	0.012 0	104	2

*Initiated approximately 6 weeks after the other male rat groups.

TABLE 2

DESIGN SUMMARY FOR B6C3F1 MICE
2,3,5,6-TETRACHLORO-4-NITROANISOLE FEEDING EXPERIMENT

	INITIAL GROUP SIZE	2,3,5,6-TETRACHLORO-4-NITROANISOLE CONCENTRATION (PERCENT)	OBSERVATION PERIOD TREATED (WEEKS)	OBSERVATION PERIOD UNTREATED (WEEKS)
MALE				
CONTROL	55	0	0	105
LOW DOSE	55	0.006 0	104	0
HIGH DOSE	55	0.012 0	104	1
FEMALE				
CONTROL	55	0	0	105
LOW DOSE	55	0.006 0	104	1
HIGH DOSE	55	0.012 0	104	1

added. These rats are referred to as the high dose male group 2, while the male members of the original high dose male rat group are referred to as high dose male group 1. The dosed rats were supplied with feed containing 2,3,5,6-tetrachloro-4-nitroanisole for a total of 104 weeks, followed by an observation period of up to 3 weeks.

All mice were approximately 6 weeks old at the time they were placed on test. The dietary concentrations of 2,3,5,6-tetrachloro-4-nitroanisole administered were 0.012 and 0.006 percent. Throughout this report those mice receiving the former concentration are referred to as the high dose groups, while those receiving the latter concentration are referred to as the low dose groups. The dosed mice were supplied with feed containing 2,3,5,6-tetrachloro-4-nitroanisole for a total of 104 weeks, followed by an observation period of up to 1 week.

G. Clinical and Histopathologic Examinations

Animals were weighed immediately prior to initiation of the experiment. Body weights were recorded twice weekly for the first 12 weeks of the study and at monthly intervals thereafter. Food consumption, for two cages from each group, was monitored for seven consecutive days once a month for the first nine months of the bioassay and for three consecutive days each month thereafter. From the first day, all animals were inspected twice daily for mortality. The presence of tissue masses and lesions was determined by monthly observation and palpation of each animal.

A necropsy was performed on each animal regardless of whether it died, was killed when moribund, or was sacrificed at the end of the bioassay. The animals were euthanized by carbon dioxide inhalation, and were immediately necropsied. The histopathologic examination consisted of gross and microscopic examination of major tissues, organs, and gross lesions taken from sacrificed animals and, whenever possible, from animals found dead.

Tissues were preserved in 10 percent buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin prior to microscopic examination. An occasional section was subjected to special staining techniques for more definitive diagnosis.

Slides were prepared from the following tissues: skin, subcutaneous tissue, lungs and bronchi, trachea, bone marrow, spleen, lymph nodes, thymus, heart, salivary gland, liver, gallbladder (mice), pancreas, esophagus, stomach, small intestine, large intestine, kidney, urinary bladder, pituitary, adrenal, thyroid, parathyroid, testis, prostate, brain, uterus, mammary gland, and ovary.

A few tissues were not examined for some animals, particularly for those that died early. Also, some animals were missing, cannibalized, or judged to be in such an advanced state of autolysis as to preclude histopathologic interpretation. Thus, the number of animals for which particular organs, tissues, or lesions were examined microscopically varies and does not necessarily represent the number of animals that were placed on experiment in each group.

H. Data Recording and Statistical Analyses

Pertinent data on this experiment have been recorded in an automatic data processing system, the Carcinogenesis Bioassay Data System (Linhart et al., 1974). The data elements include descriptive information on the chemicals, animals, experimental design, clinical observations, survival, body weight, and individual pathologic results, as recommended by the International Union Against Cancer (Berenblum, 1969). Data tables were generated for verification of data transcription and for statistical review.

These data were analyzed using the statistical techniques described in this section. Those analyses of the experimental results that bear on the possibility of carcinogenicity are discussed in the statistical narrative sections.

Probabilities of survival were estimated by the product-limit procedure of Kaplan and Meier (1958) and are presented in this report in the form of graphs. Animals were statistically censored as of the time that they died of other than natural causes or were found to be missing; animals dying from natural causes were not statistically censored. Statistical analyses for a possible dose-related effect on survival used the method of Cox (1972) when testing two groups for equality and used Tarone's (1975) extensions of Cox's methods when testing a dose-related trend. One-tailed P-values have been reported for all tests except the departure from linearity test, which is only reported when its two-tailed P-value is less than 0.05.

The incidence of neoplastic or nonneoplastic lesions has been given as the ratio of the number of animals bearing such lesions at a specific anatomic site (numerator) to the number of animals in which that site was examined (denominator). In most instances, the denominators included only those animals for which that site was examined histologically. However, when macroscopic examination was required to detect lesions prior to histologic sampling (e.g., skin or mammary tumors), or when lesions could have appeared at multiple sites (e.g., lymphomas), the denominators consist of the numbers of animals necropsied.

The purpose of the statistical analyses of tumor incidence is to determine whether animals receiving the test chemical developed a significantly higher proportion of tumors than did the control animals. As a part of these analyses, the one-tailed Fisher exact test (Cox, 1970, pp. 48-52) was used to compare the tumor incidence of a control group to that of a group of treated animals at each dose level. When results for a number of treated groups, k, are compared simultaneously with those for a control group, a correction to ensure an overall significance level of 0.05 may be made. The Bonferroni inequality (Miller, 1966, pp. 6-10) requires that the P-value for any comparison be less than or equal to 0.05/k. In cases where this correction was used, it is discussed in the narrative section. It is not, however, presented in the tables, where the Fisher exact P-values are shown.

The Cochran-Armitage test for linear trend in proportions, with continuity correction (Armitage, 1971, pp. 362-365), was also used when appropriate. Under the assumption of a linear trend, this test determined if the slope of the dose-response curve is different from zero at the one-tailed 0.05 level of significance. Unless otherwise noted, the direction of the significant trend was a positive dose relationship. This method also provides a two-tailed test of departure from linear trend.

A time-adjusted analysis was applied when numerous early deaths resulted from causes that were not associated with the formation of tumors. In this analysis, deaths that occurred before the first tumor was observed were excluded by basing the statistical tests on animals that survived at least 52 weeks, unless a tumor was found at the anatomic site of interest before week 52. When such an early tumor was found, comparisons were based exclusively on animals that survived at least as long as the animal in which the first tumor was found. Once this reduced set of data was obtained, the standard procedures for analyses of the incidence of tumors (Fisher exact tests, Cochran-Armitage tests, etc.) were followed.

When appropriate, life-table methods were used to analyze the incidence of tumors. Curves of the proportions surviving without an observed tumor were computed as in Saffiotti et al. (1972). The week during which animals died naturally or were sacrificed was entered as the time point of tumor observation. Cox's methods of comparing

these curves were used for two groups; Tarone's extension to testing for linear trend was used for three groups. The statistical tests for the incidence of tumors which used life-table methods were one-tailed and, unless otherwise noted, in the direction of a positive dose relationship. Significant departures from linearity ($P < 0.05$, two-tailed test) were also noted.

The approximate 95 percent confidence interval for the relative risk of each dosed group compared to its control was calculated from the exact interval on the odds ratio (Gart, 1971). The relative risk is defined as p_t/p_c where p_t is the true binomial probability of the incidence of a specific type of tumor in a treated group of animals and p_c is the true probability of the spontaneous incidence of the same type of tumor in a control group. The hypothesis of equality between the true proportion of a specific tumor in a treated group and the proportion in a control group corresponds to a relative risk of unity. Values in excess of unity represent the condition of a larger proportion in the treated group than in the control.

The lower and upper limits of the confidence interval of the relative risk have been included in the tables of statistical analyses. The interpretation of the limits is that in approximately 95 percent of a large number of identical experiments, the true ratio of the risk in a treated group of animals to that in a control group would be within the interval calculated from the experiment. When the lower limit of the confidence interval is greater than one, it

can be inferred that a statistically significant result (a $P < 0.025$ one-tailed test when the control incidence is not zero, $P < 0.050$ when the control incidence is zero) has occurred. When the lower limit is less than unity but the upper limit is greater than unity, the lower limit indicates the absence of a significant result while the upper limit indicates that there is a theoretical possibility of the induction of tumors by the test chemical which could not be detected under the conditions of this test.

III. CHRONIC TESTING RESULTS: RATS

A. Body Weights and Clinical Observations

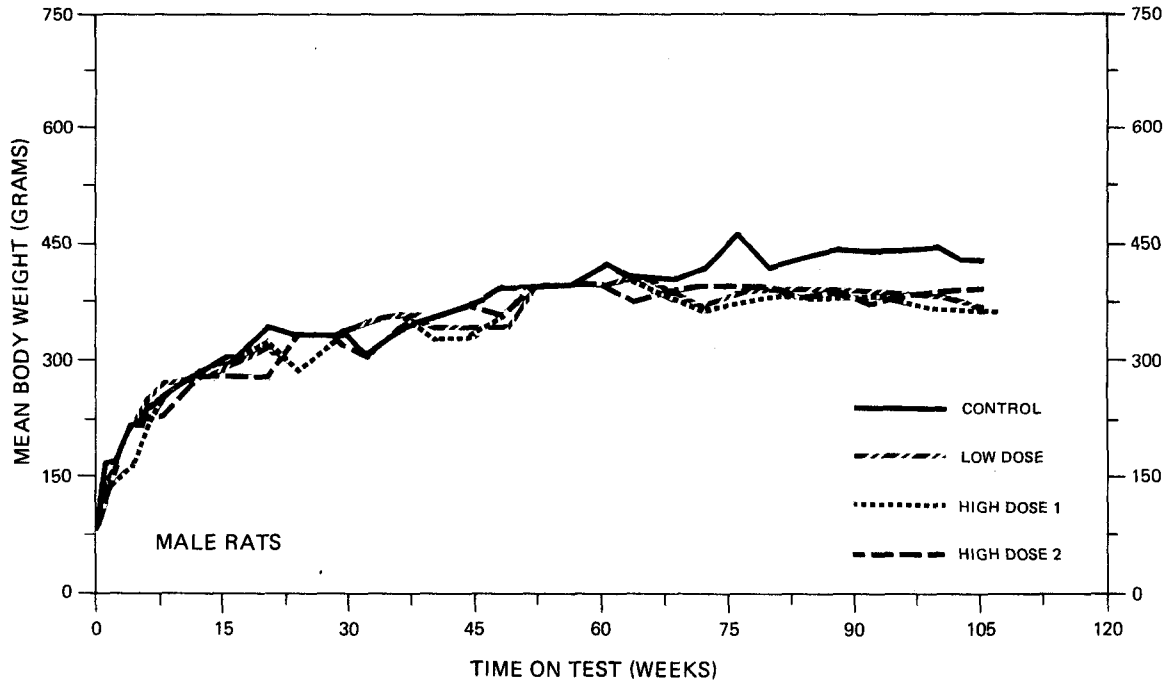
Slight mean body weight depression was observed in dosed male and female rats after 68 weeks (Figure 2).

Firm subcutaneous masses were observed in 1 control male, 7 high dose females, and 3 control females. Cutaneous lesions were observed in 2 low dose males, 3 control males, 2 high dose females, and 2 low dose females. Pale discoloration of the eyes was observed in 1 high dose male and 2 control males. Swollen, bloody eyes were observed in a second high dose male. A dark crusted eye and exudate from the nose were observed in a third high dose male. A swollen mouth was displayed by 1 high dose male. A distended scrotal sac was observed in 1 high dose male. Alopecia was observed in 1 high dose female. No other clinical abnormalities were noted.

B. Survival

The estimated probabilities of survival for male and female rats in the control and 2,3,5,6-tetrachloro-4-nitroanisole-dosed groups are shown in Figure 3. For both male and female rats no significant positive association between dosage and mortality was observed.

For each sex five control rats were sacrificed in week 78. Adequate numbers of males were at risk from late-developing tumors, as 88 percent (21/24) of high dose group 1, 68 percent (17/25) of high dose group 2, 92 percent (46/50) of the low dose group, and 72 percent



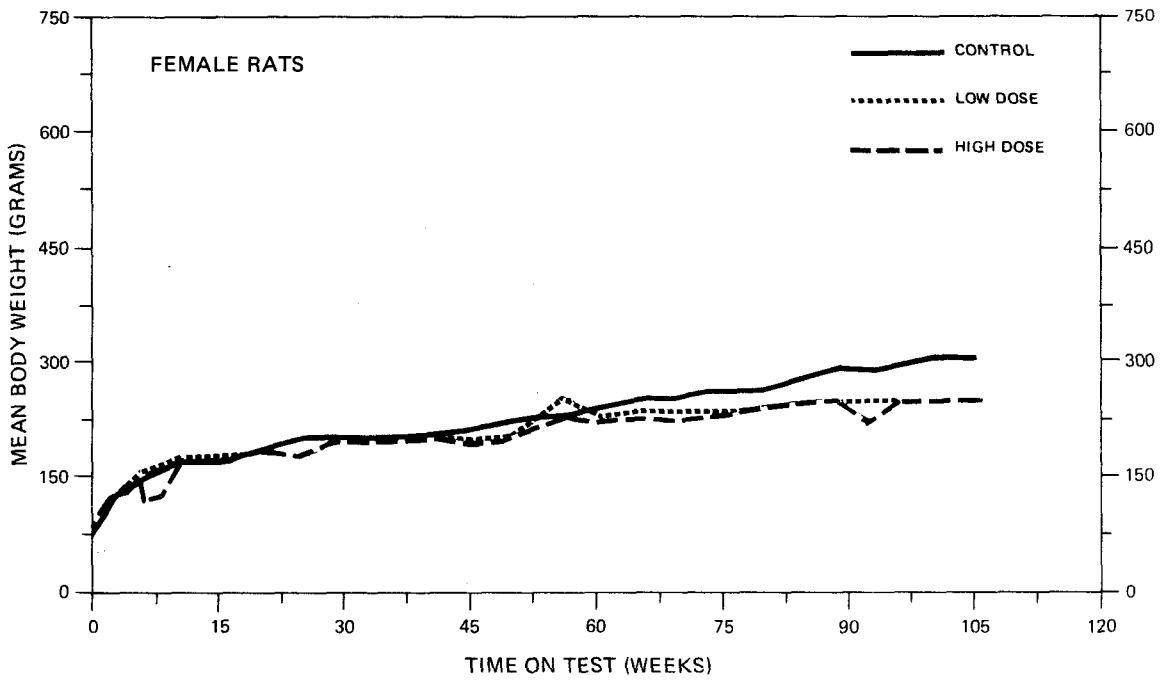
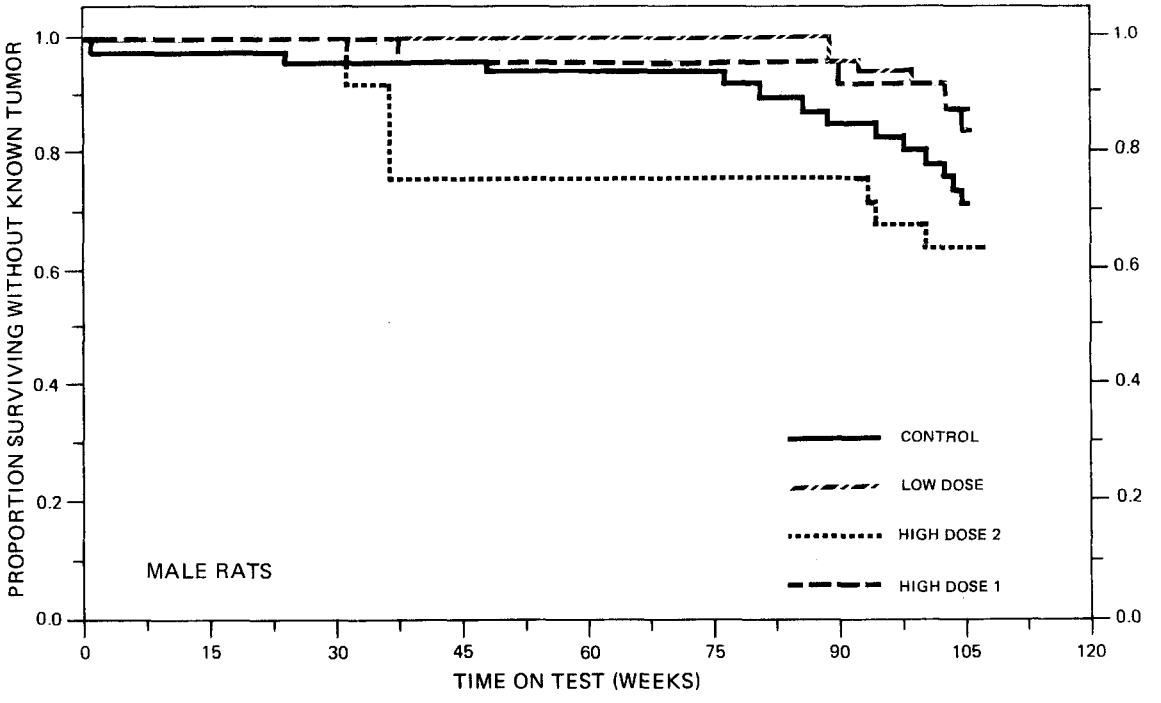


FIGURE 2
GROWTH CURVES FOR 2,3,5,6-TETRACHLORO-4-NITROANISOLE CHRONIC STUDY RATS



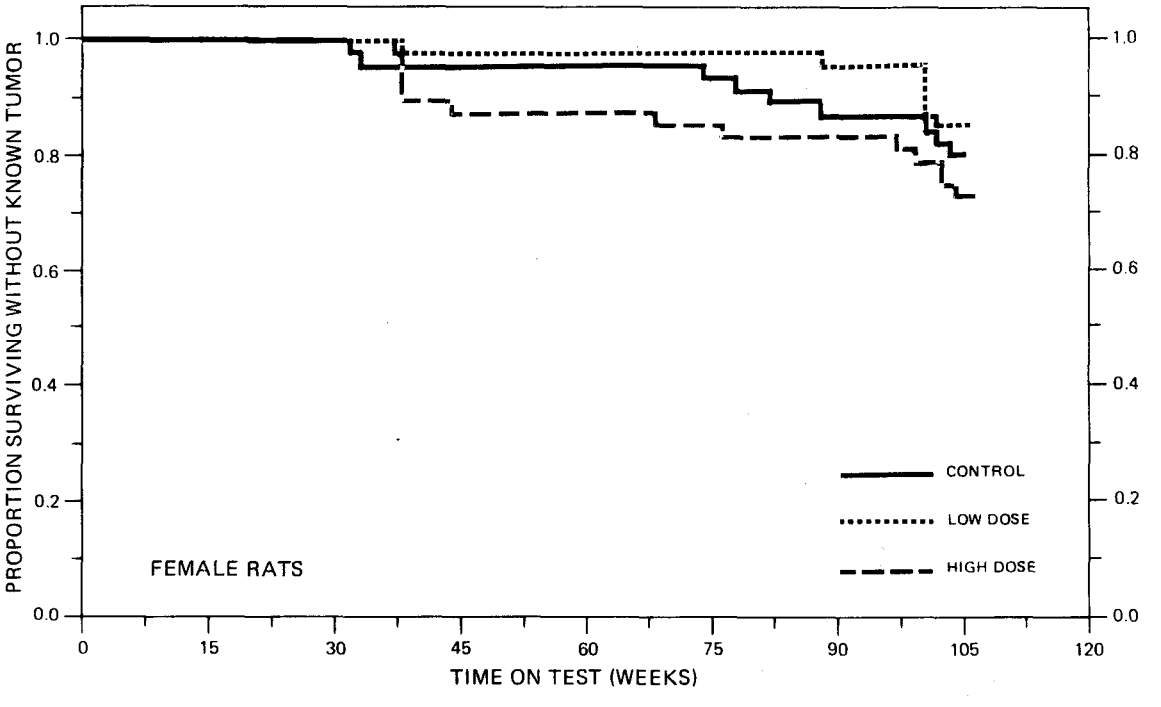


FIGURE 3
SURVIVAL COMPARISONS OF 2,3,5,6-TETRACHLORO-4-NITROANISOLE CHRONIC STUDY RATS

(36/50) of the control group survived on test for at least 100 weeks. Survival among females was also adequate as 78 percent (39/50) of the high dose, 96 percent (48/50) of the low dose, and 78 percent (39/50) of the control group survived on test at least 100 weeks.

C. Pathology

Histopathologic findings on neoplasms in rats are summarized in Appendix A (Tables A1 and A2); findings on nonneoplastic lesions are summarized in Appendix C (Tables C1 and C2).

A variety of neoplasms occurred with approximately equal frequency in dosed and control rats. These neoplasms are known to occur spontaneously in Fischer 344 rats and their distribution indicated a lack of association with chemical administration.

Hepatic neoplasms, not observed in control rats, were encountered in limited numbers among dosed rats (i.e., neoplastic nodules--3/49 [6 percent] low dose males, 1/25 [4 percent] high dose group 2 males, 0/23 high dose group 1 males, 1/50 [2 percent] low dose females, 3/45 [7 percent] high dose females; hepatocellular carcinomas--2/49 [4 percent] low dose males, 0/25 high dose group 2 males, 3/23 [13 percent] high dose group 1 males, 0/50 low dose females, 1/45 [2 percent] high dose females).

Degenerative, inflammatory, and hyperplastic lesions, frequently observed in aging Fischer 344 rats, were noted among dosed and control groups. The distribution of these nonneoplastic lesions did not provide evidence for an association with chemical administration.

Occasional lesions appeared to be sex-related (e.g. chronic nephropathy in males), and these findings were compatible with the incidences observed in historical controls.

Based upon this histopathologic examination, convincing evidence was not provided for the carcinogenicity of dietary administration of 2,3,5,6-tetrachloro-4-nitroanisole; however, there was an increased incidence of hepatic lesions in both sexes.

D. Statistical Analyses of Results

The results of the statistical analyses of tumor incidence in rats are summarized in Tables 3 and 4. The analysis is included for every type of malignant tumor in either sex where at least two such tumors were observed in at least one of the control or 2,3,5,6-tetrachloro-4-nitroanisole-dosed groups and where such tumors were observed in at least 5 percent of the group. In these analyses the Cochran-Armitage test was not used for high dose group 2 since this group was started on test approximately 6 weeks after all the other groups.

For male rats the Cochran-Armitage test indicated a significant ($P = 0.020$) positive association between dosage and the combined incidence of hepatocellular carcinomas and neoplastic nodules of the liver when high dose group 1 was used. None of the Fischer exact test results were significant, however, under the Bonferroni criterion. Similarly, for liver neoplasms in the females the Cochran-Armitage test was significant ($P = 0.020$), but the Fisher exact tests were not

TABLE 3

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT SPECIFIC SITES IN MALE RATS TREATED WITH 2,3,5,6-TETRACHLORO-4-NITROANISOLE[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE GROUP 2	HIGH DOSE GROUP 1
Subcutaneous Tissue: Fibroma[b]	3/48(0.06)	2/49(0.04)	0/25(0.00)	0/23(0.00)
P Values[c]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.653	0.000	0.000
Lower Limit	---	0.055	0.000	0.000
Upper Limit	---	5.345	3.111	3.366
Weeks to First Observed Tumor	105	105	---	---
Lung: Alveolar/Bronchiolar Adenoma[b]	3/48(0.06)	0/49(0.00)	1/25(0.04)	0/23(0.00)
P Values[c]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.000	0.640	0.000
Lower Limit	---	0.000	0.012	0.000
Upper Limit	---	1.628	7.396	3.366
Weeks to First Observed Tumor	105	---	106	---
Hematopoietic System: Leukemia or Malignant Lymphoma[b]	7/48(0.15)	7/49(0.14)	3/25(0.12)	6/23(0.26)
P Values[c]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.980	0.823	1.789
Lower Limit	---	0.311	0.147	0.550
Upper Limit	---	2.969	3.217	5.356
Weeks to First Observed Tumor	80	92	36	89

TABLE 3 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE GROUP 2	HIGH DOSE GROUP 1
Liver: Hepatocellular Carcinoma[b]	0/48(0.00)	2/49(0.04)	0/25(0.00)	3/23(0.13)
P Values[c]	P = 0.015	N.S.	N.S.	P = 0.031
Relative Risk (Control)[d]	---	Infinite	---	Infinite
Lower Limit	---	0.290	---	1.265
Upper Limit	---	Infinite	---	Infinite
Weeks to First Observed Tumor	---	105	---	105
Liver: Hepatocellular Carcinoma or Neoplastic Nodule[b]	0/48(0.00)	5/49(0.10)	1/25(0.04)	3/23(0.13)
P Values[c]	P = 0.020	P = 0.030	N.S.	P = 0.031
Relative Risk (Control)[d]	---	Infinite	Infinite	Infinite
Lower Limit	---	1.237	0.103	1.265
Upper Limit	---	Infinite	Infinite	Infinite
Weeks to First Observed Tumor	---	105	106	105
Pituitary: Adenoma NOS[b]	10/45(0.22)	9/43(0.21)	4/21(0.19)	4/20(0.20)
P Values[c]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.942	0.857	0.900
Lower Limit	---	0.375	0.216	0.227
Upper Limit	---	2.320	2.543	2.651
Weeks to First Observed Tumor	102	103	106	105

TABLE 3 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE GROUP 2	HIGH DOSE GROUP 1
Adrenal: Pheochromocytoma[b]	4/46(0.09)	4/49(0.08)	3/25(0.12)	4/22(0.18)
P Values[c]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.939	1.380	2.091
Lower Limit	---	0.185	0.215	0.421
Upper Limit	---	4.761	7.405	9.986
Weeks to First Observed Tumor	78	103	106	105
Thyroid: C-Cell Carcinoma or C-Cell Adenoma[b]	5/43(0.12)	2/45(0.04)	0/22(0.00)	2/22(0.09)
P Values[c]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.382	0.000	0.782
Lower Limit	---	0.038	0.000	0.078
Upper Limit	---	2.194	1.496	4.277
Weeks to First Observed Tumor	94	103	---	105
Pancreatic Islets: Islet-Cell Adenoma or Islet-Cell Carcinoma[b]	0/44(0.00)	2/46(0.04)	1/25(0.04)	2/21(0.10)
P Values[c]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	Infinite	Infinite	Infinite
Lower Limit	---	0.284	0.094	0.625
Upper Limit	---	Infinite	Infinite	Infinite
Weeks to First Observed Tumor	---	105	106	105

TABLE 3 (CONCLUDED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE GROUP 2	HIGH DOSE GROUP 1
Testis: Interstitial-Cell Tumor[b]	37/48(0.77)	46/49(0.94)	19/24(0.79)	22/23(0.96)
P Values[c]	P = 0.009	P = 0.018	N.S.	P = 0.046
Relative Risk (Control)[d]	---	1.218	1.027	1.241
Lower Limit	---	1.011	0.745	0.968
Upper Limit	---	1.364	1.302	1.331
Weeks to First Observed Tumor	78	92	36	103

[a]Treated groups received doses of 0.006 or 0.012 percent in feed.

[b]Number of tumor-bearing animals/number of animals examined at site (proportion).

[c]The probability level for the Cochran-Armitage test for the comparison of the control group and the low dose group with high dose Group 1 is given beneath the incidence of tumors in the control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. The probability level for the Fisher exact test for the comparison of a treated group with the control group is given beneath the incidence of tumors in that treated group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. For both Cochran-Armitage and Fisher exact tests a negative designation (N) indicates a lower incidence in the treated group(s) than in the control group.

[d]The 95% confidence interval on the relative risk of the treated group to the control group.

TABLE 4

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT SPECIFIC SITES IN FEMALE RATS TREATED WITH 2,3,5,6-TETRACHLORO-4-NITROANISOLE[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Subcutaneous Tissue: Fibroadenoma[b]	0/50(0.00)	4/50(0.08)	0/45(0.00)
P Values[c]	N.S.	N.S.	N.S.
Departure from Linear Trend[e]	P = 0.006	---	---
Relative Risk (Control)[d]	---	Infinite	---
Lower Limit	---	0.927	---
Upper Limit	---	Infinite	---
Weeks to First Observed Tumor	---	100	---
Hematopoietic System: Leukemia or Malignant Lymphoma[b]	4/50(0.08)	7/50(0.14)	7/45(0.16)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.750	1.944
Lower Limit	---	0.476	0.531
Upper Limit	---	7.682	8.487
Weeks to First Observed Tumor	101	88	97
Liver: Hepatocellular Carcinoma or Neoplastic Nodule[b]	0/50(0.00)	1/50(0.02)	4/45(0.09)
P Values[c]	P = 0.020	N.S.	P = 0.047
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.054	1.031
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	105	105

TABLE 4 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Pituitary: Adenoma NOS[b]	16/40(0.40)	15/48(0.31)	14/40(0.35)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.781	0.875
Lower Limit	---	0.418	0.462
Upper Limit	---	1.470	1.641
Weeks to First Observed Tumor	100	105	102
Pituitary: Adenoma NOS or Chromophobe Adenoma[b]	17/40(0.43)	15/48(0.31)	14/40(0.35)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.735	0.824
Lower Limit	---	0.399	0.441
Upper Limit	---	1.360	1.518
Weeks to First Observed Tumor	100	105	102
Pituitary: Adenoma NOS or Carcinoma NOS[b]	16/40(0.40)	16/48(0.33)	15/40(0.38)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.833	0.938
Lower Limit	---	0.455	0.506
Upper Limit	---	1.547	1.729
Weeks to First Observed Tumor	100	105	102

TABLE 4 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Adrenal: Pheochromocytoma[b]	6/48(0.13)	1/50(0.02)	0/44(0.00)
P Values[c]	P = 0.006(N)	N.S.	P = 0.017(N)
Relative Risk (Control)[d]	---	0.160	0.000
Lower Limit	---	0.004	0.000
Upper Limit	---	1.249	0.679
Weeks to First Observed Tumor	105	105	---
Thyroid: C-Cell Adenoma[b]	0/43(0.00)	3/47(0.06)	0/37(0.00)
P Values[c]	N.S.	N.S.	N.S.
Departure from Linear Trend[e]	P = 0.022	---	---
Relative Risk (Control)[d]	---	Infinite	---
Lower Limit	---	0.553	---
Upper Limit	---	Infinite	---
Weeks to First Observed Tumor	---	88	---
Thyroid: C-Cell Adenoma or C-Cell Carcinoma[b]	0/43(0.00)	4/47(0.09)	1/37(0.03)
P Values[c]	N.S.	N.S.	N.S.
Departure from Linear Trend[e]	P = 0.045	---	---
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.852	0.062
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	88	106

TABLE 4 (CONCLUDED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Mammary Gland: Fibroadenoma[b]	6/50(0.12)	0/50(0.00)	1/45(0.02)
P Values[c]	P = 0.021(N)	P = 0.013(N)	N.S.
Relative Risk (Control)[d]	---	0.000	0.185
Lower Limit	---	0.000	0.004
Upper Limit	---	0.625	1.441
Weeks to First Observed Tumor	105	---	106
Uterus: Endometrial Stromal Polyp[b]	2/48(0.04)	9/50(0.18)	8/45(0.18)
P Values[c]	P = 0.036	P = 0.030	P = 0.036
Relative Risk (Control)[d]	---	4.320	4.267
Lower Limit	---	0.957	0.911
Upper Limit	---	39.430	39.438
Weeks to First Observed Tumor	105	105	102

[a]Treated groups received doses of 0.006 or 0.012 percent in feed.

[b]Number of tumor-bearing animals/number of animals examined at site (proportion).

[c]The probability level for the Cochran-Armitage test is given beneath the incidence of tumors in the control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. The probability level for the Fisher exact test for the comparison of a treated group with the control group is given beneath the incidence of tumors in the treated group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. For both Cochran-Armitage and Fisher exact tests a negative designation (N) indicates a lower incidence in the treated group(s) than in the control group.

[d]The 95% confidence interval on the relative risk of the treated group to the control group.

[e]The probability level of the test for departure from linear trend is given beneath the control group when $P < 0.05$.

significant under the Bonferroni criterion. In historical control data collected by this laboratory for the NCI Carcinogenesis Testing Program 6/250 (2 percent) of the male and 35/249 (14 percent) of the female untreated Fischer 344 rats had one of these tumors. For endometrial stromal polyps in the females the Cochran-Armitage test was significant, but the Fisher exact tests were not under the Bonferroni criterion. In historical control data 31/249 (12 percent) of the untreated females had one of these tumors.

Significant positive associations were observed for interstitial-cell tumors of the testis in males, but these results must be discounted due to the usually high spontaneous incidence of this tumor (Cockrell and Garner, 1976).

For females the possibility of a negative association between dosage and incidence was observed for adrenal pheochromocytomas and for mammary fibroadenomas.

To provide additional insight into the possible carcinogenicity of this compound, 95 percent confidence intervals on the relative risk have been estimated and entered in the tables based upon the observed tumor incidence rates. In many of the intervals shown in Tables 3 and 4, the value one is included; this indicates the absence of statistically significant results. It should also be noted that many of the confidence intervals have an upper limit greater than one, indicating the theoretical possibility of tumor induction in rats by 2,3,5,6-tetrachloro-4-nitroanisole that could not be established under the conditions of this test.

IV. CHRONIC TESTING RESULTS: MICE

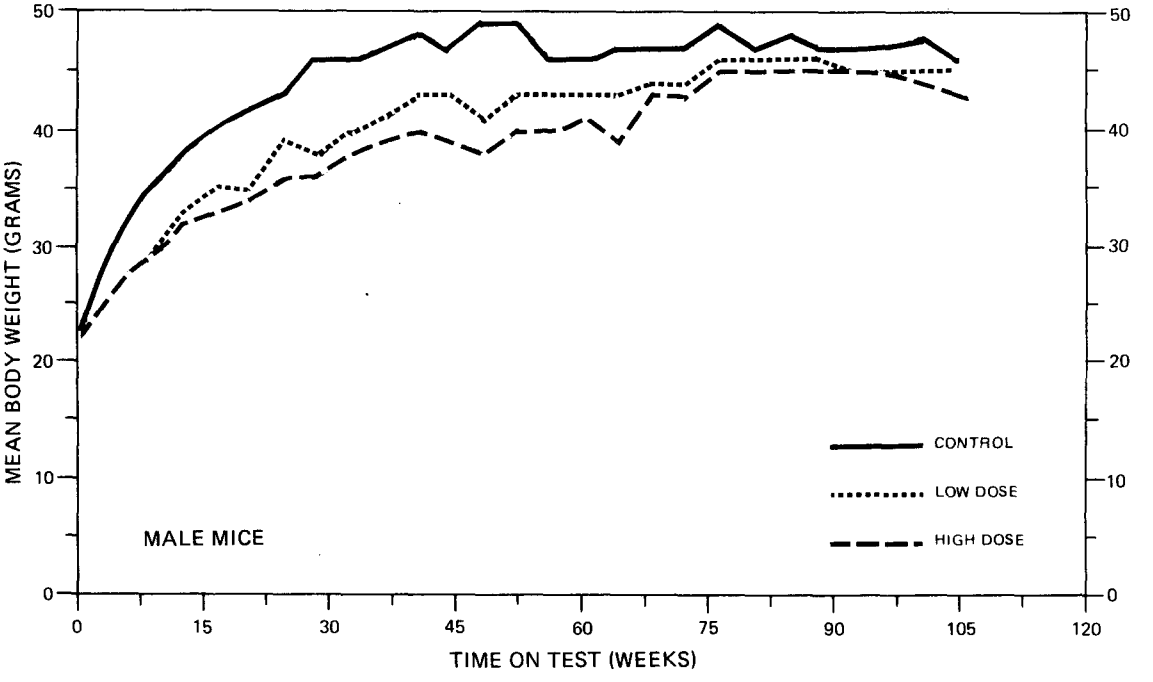
A. Body Weights and Clinical Observations

Mean body weight depression, observed in both dosed male and dosed female mice when compared to controls, was more apparent during the first year of study for males and during the second year of study for females (Figure 4).

Abdominal distention, with and without palpable masses, was observed in 2 high dose males, 5 low dose females, and 1 control female. Swelling of the urogenital or rectal area was observed in 1 high dose male, 2 low dose males, 1 control male, and 1 high dose female. Blood in the urogenital area was observed in 2 control males. Subcutaneous masses were observed in 4 control males, 2 low dose females, and 2 control females. Swollen eyes were noted in 2 high dose females, 1 low dose female, and 1 control male; in this male a mass developed in the Harderian gland. Cutaneous lesions were observed in 2 high dose males, 3 low dose males, 1 control male, 1 high dose female, 1 low dose female, and 1 control female. Alopecia was observed in 17 high dose males, 20 low dose males, 42 control males, 25 high dose females, 23 low dose females, and 54 control females. No other clinical abnormalities were noted.

B. Survival

The estimated probabilities of survival for male and female mice in the control and 2,3,5,6-tetrachloro-4-nitroanisole-dosed groups are shown in Figure 5. For both male and female mice there was no significant positive association between dosage and mortality.



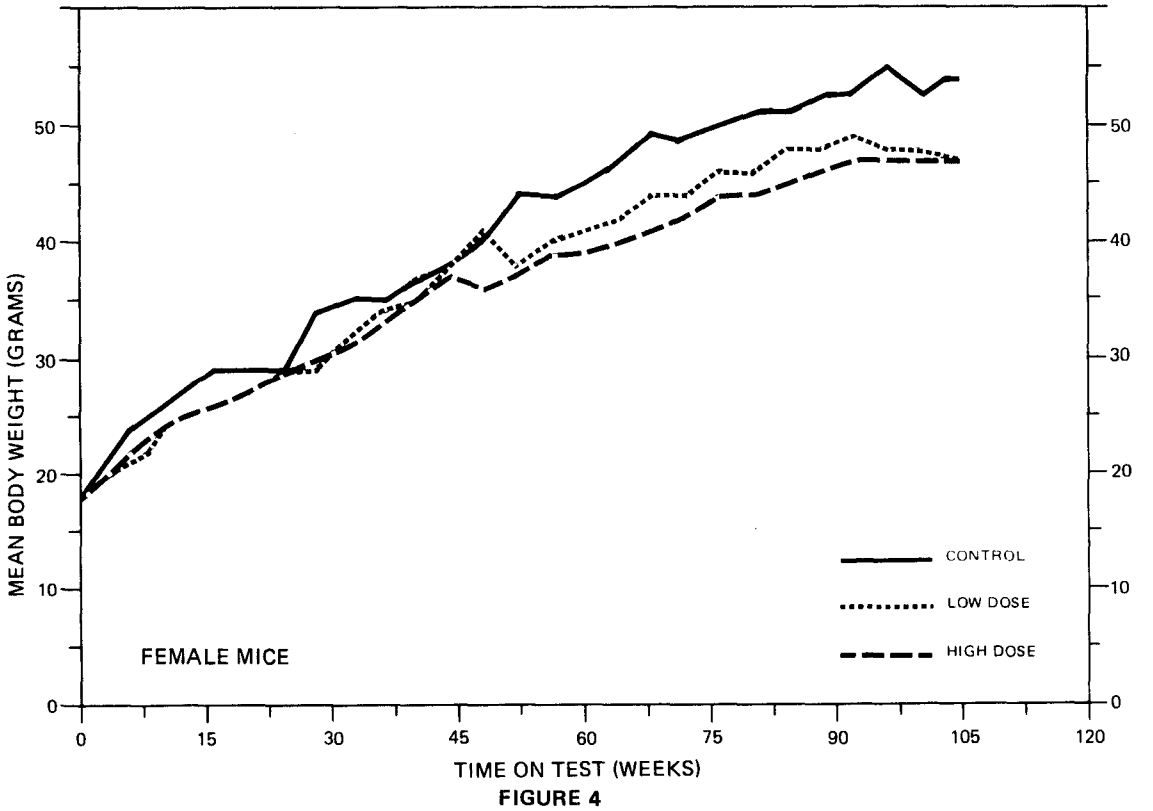
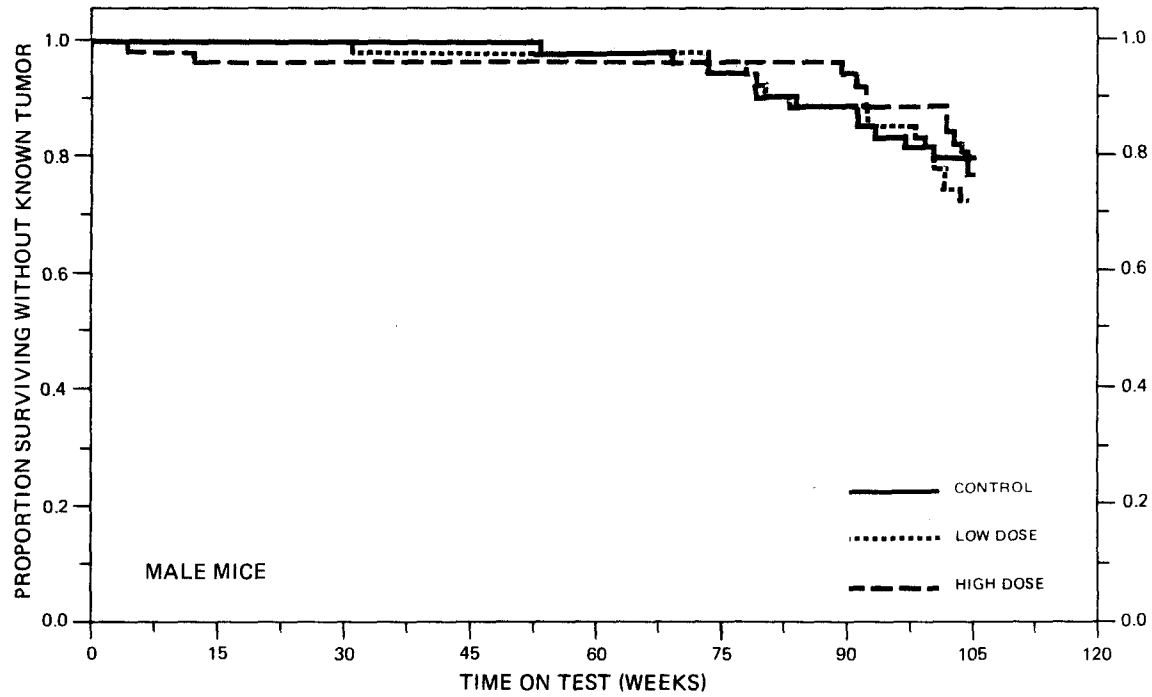


FIGURE 4
GROWTH CURVES FOR 2,3,5,6-TETRACHLORO-4-NITROANISOLE CHRONIC STUDY MICE



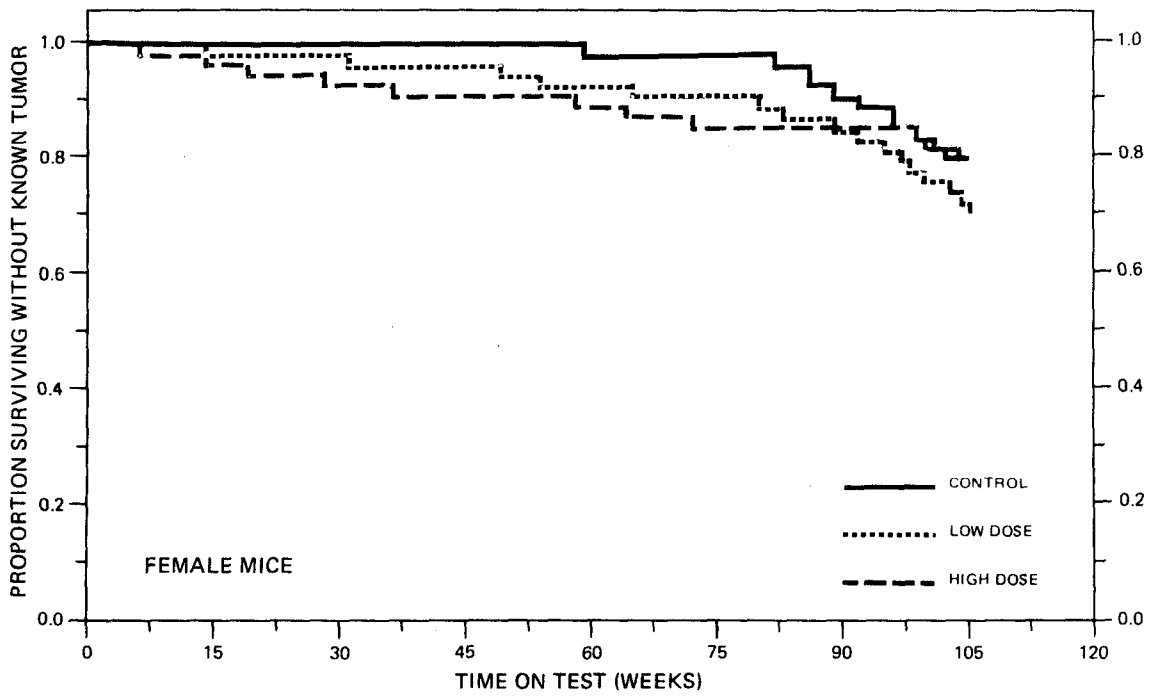


FIGURE 5
SURVIVAL COMPARISONS OF 2,3,5,6-TETRACHLORO-4-NITROANISOLE CHRONIC STUDY MICE

Adequate numbers of males were at risk from late-developing tumors as 91 percent (50/55) of the high dose, 89 percent (49/55) of the low dose, and 89 percent (49/55) of the control group survived on test at least 85 weeks. Survival among the females was also adequate as 84 percent (46/55) of the high dose, 85 percent (47/55) of the low dose, and 96 percent (53/55) of the control group survived on test for at least 85 weeks.

C. Pathology

Histopathologic findings on neoplasms in mice are summarized in Appendix B (Tables B1 and B2); findings on nonneoplastic lesions are summarized in Appendix D (Tables D1 and D2).

A dose-related increase in the incidence of malignant lymphomas was observed among dosed mice as shown in the following table:

	Males			Females		
	Control	Low Dose	High Dose	Control	Low Dose	High Dose
No. of Animals Necropsied	(55)	(55)	(53)	(55)	(54)	(52)
Malignant Lymphoma NOS	0	3	0	2	0	1
Malignant Lymphoma, Undifferentiated	0	1	5	0	2	4
Malignant Lymphoma, Lymphocytic	0	4	2	2	15	13
Malignant Lymphoma, Histiocytic	1	0	1	1	4	0
Malignant Lymphoma, Mixed	3	0	4	9	4	7
Lymphocytic Leukemia	0	0	0	4	0	0
Granulocytic Leukemia	0	1	0	0	0	0
Total number of animals with lymphomas or leukemias	4	9	12	18	25	25

These data suggest that administration of 2,3,5,6-tetrachloro-4-nitroanisole may have induced lymphomas, particularly in male mice.

The malignant lymphomas were classified as lymphocytic, histiocytic, mixed, or undifferentiated. The cell types were characterized as follows:

> Lymphocytic: Round, basophilic lymphocytes with little or no cytoplasm, and often resembling normal lymphocytes. A moderate degree of differentiation was usual.
>
> Histiocytic: Round, ovoid, sometimes indented nuclei surrounded by abundant, granular pink cytoplasm.
>
> Mixed: A combination of lymphocytic and histiocytic cells. The histiocytic tumor cells sometimes folded and occasionally formed giant cells.
>
> Undifferentiated: A uniform population of "blast" type cells with large, pale nuclei and, commonly, a single nucleolus. Cytoplasmic boundaries were indistinct.

Lymphomas with circulating malignant cells were termed "leukemic" or lymphocytic leukemia.

A variety of other commonly occurring neoplasms was encountered among dosed and control groups of both sexes. The incidence of these neoplasms indicated that they were not associated with chemical administration.

Acanthosis, hyperkeratosis, or both were detected in the forestomach in 4/52 (8 percent) low dose and 5/50 (10 percent) high dose males; and in 4/51 (8 percent) low dose and 7/51 (14 percent) high dose females. Although few in number, the distribution of these lesions, when compared to that in historical control mice, indicates

an association with chemical administration. There was no apparent progression of these lesions to neoplasia.

In male mice the number of animals with hepatocellular lesions was highest in the control group, less in the low dose, and least in the high dose group.

Based upon this histopathologic examination, administration of 2,3,5,6-tetrachloro-4-nitroanisole to mice was associated with a slightly increased incidence of malignant lymphomas, particularly in males. A slight increase in the incidence of acanthosis and hyperkeratosis of the forestomach was noted in dosed mice of both sexes.

D. Statistical Analyses of Results

The results of the statistical analyses of tumor incidence in mice are summarized in Tables 5 and 6. The analysis is included for every type of malignant tumor in either sex where at least two such tumors were observed in at least one of the control or 2,3,5,6-tetrachloro-4-nitroanisole-dosed groups and where such tumors were observed in at least 5 percent of the group.

For male mice the Cochran-Armitage test indicated a significant ($P = 0.019$) positive association between dosage and the combined incidence of leukemia or malignant lymphomas. The Fisher exact tests supported this result with a significant ($P = 0.023$) comparison of high dose to control. For females no statistical tests were significant although leukemia or malignant lymphoma was observed in 25/52 (48 percent) of the high dose mice. In historical control data

TABLE 5

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT SPECIFIC SITES IN MALE MICE TREATED WITH 2,3,5,6-TETRACHLORO-4-NITROANISOLE[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Subcutaneous Tissue: Fibrosarcoma or Leiomyosarcoma[b]	1/55(0.02)	3/55(0.05)	2/53(0.04)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	3.000	2.075
Lower Limit	---	0.250	0.111
Upper Limit	---	154.535	120.111
Weeks to First Observed Tumor	73	103	101
Lung: Alveolar/Bronchiolar Carcinoma[b]	6/54(0.11)	13/54(0.24)	9/51(0.18)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	2.167	1.588
Lower Limit	---	0.835	0.545
Upper Limit	---	6.445	5.043
Weeks to First Observed Tumor	105	78	105
Lung: Alveolar/Bronchiolar Carcinoma or Alveolar/Bronchiolar Adenoma[b]	12/54(0.22)	17/54(0.31)	13/51(0.25)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.417	1.147
Lower Limit	---	0.709	0.534
Upper Limit	---	2.922	2.482
Weeks to First Observed Tumor	79	78	89

TABLE 5 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Hematopoietic System: Leukemia or Malignant Lymphoma[b]	4/55(0.07)	9/55(0.16)	12/53(0.23)
P Values[c]	P = 0.019	N.S.	P = 0.023
Relative Risk (Control)[d]	---	2.250	3.113
Lower Limit	---	0.672	1.016
Upper Limit	---	9.455	12.452
Weeks to First Observed Tumor	105	73	91
Liver: Hepatocellular Carcinoma[b]	24/54(0.44)	13/52(0.25)	9/52(0.17)
P Values[c]	P = 0.002(N)	P = 0.029(N)	P = 0.002(N)
Relative Risk (Control)[d]	---	0.563	0.389
Lower Limit	---	0.299	0.179
Upper Limit	---	1.015	0.775
Weeks to First Observed Tumor	53	79	92
Liver: Hepatocellular Carcinoma, Hepatocellular Adenoma, or Mixed Hepato/Cholangio Carcinoma[b]	28/54(0.52)	18/52(0.35)	12/52(0.23)
P Values[c]	P = 0.002(N)	N.S.	P = 0.002(N)
Relative Risk (Control)[d]	---	0.668	0.445
Lower Limit	---	0.405	0.237
Upper Limit	---	1.083	0.795
Weeks to First Observed Tumor	53	79	92

TABLE 5 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Liver: Angiosarcoma[b]	0/54(0.00)	2/52(0.04)	3/52(0.06)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.307	0.623
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	97	105
Adrenal: Capsular Adenoma NOS or Cortical Adenoma[b]	6/50(0.12)	0/52(0.00)	0/49(0.00)
P Values[c]	P = 0.003(N)	P = 0.012(N)	P = 0.014(N)
Departure from Linear Trend[e]	P = 0.070	---	---
Relative Risk (Control)[d]	---	0.000	0.000
Lower Limit	---	0.000	0.000
Upper Limit	---	0.602	0.637
Weeks to First Observed Tumor	105	---	---
Thyroid: Follicular-Cell Adenoma or Follicular-Cell Carcinoma[b]	0/48(0.00)	3/49(0.06)	0/44(0.00)
P Values[c]	N.S.	N.S.	N.S.
Departure from Linear Trend[e]	P = 0.017	---	---
Relative Risk (Control)[d]	---	Infinite	---
Lower Limit	---	0.590	---
Upper Limit	---	Infinite	---
Weeks to First Observed Tumor	---	104	---

TABLE 5 (CONCLUDED)

[a]Treated groups received doses of 0.006 or 0.012 percent in feed.

[b]Number of tumor-bearing animals/number of animals examined at site (proportion).

[c]The probability level for the Cochran-Armitage test is given beneath the incidence of tumors in the control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. The probability level for the Fisher exact test for the comparison of a treated group with the control group is given beneath the incidence of tumors in the treated group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. For both Cochran-Armitage and Fisher exact tests a negative designation (N) indicates a lower incidence in the treated group(s) than in the control group.

[d]The 95% confidence interval on the relative risk of the treated group to the control group.

[e]The probability level of the test for departure from linear trend is given beneath the control group when $P < 0.05$.

TABLE 6

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT SPECIFIC SITES IN FEMALE MICE TREATED WITH 2,3,5,6-TETRACHLORO-4-NITROANISOLE[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Lung: Alveolar/Bronchiolar Carcinoma[b]	1/55(0.02)	5/52(0.10)	2/52(0.04)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	5.289	2.115
Lower Limit	---	0.620	0.114
Upper Limit	---	244.998	122.378
Weeks to First Observed Tumor	105	97	72
Lung: Alveolar/Bronchiolar Carcinoma or Alveolar/Bronchiolar Adenoma[b]	4/55(0.07)	8/52(0.15)	4/52(0.08)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	2.115	1.058
Lower Limit	---	0.606	0.207
Upper Limit	---	9.064	5.393
Weeks to First Observed Tumor	105	83	72
Hematopoietic System: Leukemia or Malignant Lymphoma[b]	18/55(0.33)	25/54(0.46)	25/52(0.48)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.415	1.469
Lower Limit	---	0.847	0.882
Upper Limit	---	2.392	2.470
Weeks to First Observed Tumor	86	80	64

TABLE 6 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Liver: Hepatocellular Carcinoma[b]	7/54(0.13)	1/53(0.02)	3/52(0.06)
P Values[c]	N.S.	P = 0.032(N)	N.S.
Relative Risk (Control)[d]	---	0.146	0.445
Lower Limit	---	0.003	0.078
Upper Limit	---	1.076	1.832
Weeks to First Observed Tumor	101	105	105
Liver: Hepatocellular Carcinoma, Hepatocellular Adenoma or Mixed Hepato/Cholangio Carcinoma[b]	11/54(0.20)	4/53(0.08)	5/52(0.10)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.370	0.472
Lower Limit	---	0.091	0.138
Upper Limit	---	1.161	1.363
Weeks to First Observed Tumor	59	105	105
Pituitary: Adenoma NOS[b]	0/42(0.00)	2/47(0.04)	3/41(0.07)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.266	0.620
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	105	105

TABLE 6 (CONCLUDED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Pituitary: Adenoma NOS, Chromophobe Adenoma, or Basophil Adenoma[b]	3/42(0.07)	2/47(0.04)	3/41(0.07)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.596	1.024
Lower Limit	---	0.052	0.145
Upper Limit	---	4.956	7.232
Weeks to First Observed Tumor	105	105	105

[a]Treated groups received doses of 0.006 or 0.012 percent in feed.

[b]Number of tumor-bearing animals/number of animals examined at site (proportion).

[c]The probability level for the Cochran-Armitage test is given beneath the incidence of tumors in the control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. The probability level for the Fisher exact test for the comparison of a treated group with the control group is given beneath the incidence of tumors in the treated group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. For both Cochran-Armitage and Fisher exact tests a negative designation (N) indicates a lower incidence in the treated group(s) than in the control group.

[d]The 95% confidence interval on the relative risk of the treated group to the control group.

collected by this laboratory for the NCI Carcinogenesis Testing Program, leukemia or malignant lymphoma was detected in 22/259 (8 percent) of the untreated control male and 44/270 (16 percent) of the untreated control female B6C3F1 mice. Of the 6 groups of untreated control male mice and the 6 groups of untreated control female mice included in these historical control incidences, the highest incidence of this combination of neoplasms was 11/39 (28 percent) for males and 11/50 (22 percent) for females.

For male mice the possibility of a negative association between dosage and the incidences of hepatocellular carcinomas and of adenomas NOS of the adrenal was noted. No other statistical tests for either sex were significant under the Bonferroni criterion.

To provide additional insight into the possible carcinogenicity of this compound, 95 percent confidence intervals on the relative risk have been estimated and entered in the tables based upon the observed tumor incidence rates. In many of the intervals shown in Tables 5 and 6, the value one is included; this indicates the absence of statistically significant results. It should also be noted that many of the confidence intervals have an upper limit greater than one, indicating the theoretical possibility of tumor induction in mice by 2,3,5,6-tetrachloro-4-nitroanisole that could not be established under the conditions of this test.

V. DISCUSSION

There were no significant positive associations between the dietary concentration of 2,3,5,6-tetrachloro-4-nitroanisole administered and mortality in rats or mice of either sex. Adequate numbers of animals in all groups survived sufficiently long to be at risk from late-developing tumors.

Although the incidences of interstitial-cell testicular tumors in dosed male rats were statistically significantly higher than in controls, this was discounted due to the high and variable spontaneous incidence of this lesion in Fischer 344 rats (Cockrell and Garner, 1976). There was an association between dosage and the combined incidence of hepatocellular carcinomas and neoplastic nodules in both male and female rats. However, neither these tumors nor any other tumors occurred at significantly increased incidences when dosed male or female rats were compared to their controls.

When those male mice having either leukemia or malignant lymphoma were combined and the resulting incidences analyzed, the Cochran-Armitage test indicated a significant positive association between the concentration of the compound administered and occurrence of these neoplasms. The high dose to control Fisher exact comparison supported the finding. These hematopoietic lesions occur spontaneously and with great variation in B6C3F1 mice (i.e., historical control incidences of 8 and 16 percent for untreated control males and females, respectively, with maximum incidences in one group of 28 and 22 percent for

males and females, respectively); therefore, the administration of the compound was not considered to be associated with their development. No other tumors occurred in significant positive incidences when dosed mice of either sex were compared to controls. There was a negative trend for the incidences of hepatocellular carcinomas in male mice, which was not attributable to poor survival among the dosed groups.

Under the conditions of this bioassay, dietary administration of 2,3,5,6-tetrachloro-4-nitroanisole was not carcinogenic to either sex of Fischer 344 rats or to B6C3F1 mice.

VI. BIBLIOGRAPHY

Armitage, P., Statistical Methods in Medical Research, Chapter 14. J. Wiley & Sons, New York, 1971.

Berckmans, V.S.F. and A.F. Halleman, Recueil des travaux Chimiques des Pays-bas 44:851, 1925.

Berenblum, I., editor, Carcinogenicity Testing. International Union Against Cancer, Technical Report Series, Vol. 2. International Union Against Cancer, Geneva, 1969.

Carey, W.F., "Determination of Tetrachloronitroanisole (TCNA) Residues in Vegetables and Grains." Journal of the Association of Official Analytical Chemists 46:876-878, 1963.

Chemical Abstracts Service. The Chemical Abstracts Service (CAS) Ninth Collective Index, Volumes 76-85, 1972-1976. American Chemical Society, Washington, D.C., 1977.

Cockrell, B.Y. and F.M. Garner, "Interstitial-Cell Tumors of the Testis in Rats." Comparative Pathology Bulletin 8:2-4, 1976.

Cox, D.R., Analysis of Binary Data, Chapters 4 and 5. Methuen and Co., Ltd., London, 1970.

Cox, D.R., "Regression Models and Life-Tables." Journal of the Royal Statistical Society, Series "B" 34:187-220, 1972.

Gart, J.J., "The Comparison of Proportions: A Review of Significance Tests, Confidence Limits, and Adjustments for Stratification." International Statistical Institute Review 39:148-169, 1971.

Kaplan, E.L., and P. Meier, "Nonparametric Estimation from Incomplete Observations." Journal of the American Statistical Association 53:457-481, 1958.

Linhart, M.S., J.A. Cooper, R.L. Martin, N.P. Page, and J.A. Peters, "Carcinogenesis Bioassay Data System." Computers and Biomedical Research 7:230-248, 1974.

Miller, R.G., Simultaneous Statistical Inference. McGraw-Hill Book Co., New York, 1966.

Peters, A.T., R.M. Rocoe, and D.M. Stead, Journal of the Chemical Society, 233, 1943.

Purdy, L.H., "Comparative Effectiveness of Seed-Treatment Chemicals Against Flag Smut of Wheat." Plant Disease Reporter 47(9):793-796, 1963.

Saffiotti, U., R. Montesano, A.R. Sellakumar, F. Cefis, and D.G. Kaufman, "Respiratory Tract Carcinogenesis in Hamsters Induced by Different Numbers of Administration of Benzo (a) Pyrene and Ferric Oxide." Cancer Research 32:1073-1079, 1972.

Schaughnessy, J., Registration Division, U.S. Environmental Protection Agency, Washington, D.C. Personal communication, May 24, 1977.

Tarone, R.E., "Tests for Trend in Life-Table Analysis." Biometrika 62:679-682, 1975.

U.S. Department of Health, Education, and Welfare, Report of the Secretary's Commission on Pesticides and their Relationship to Environmental Health. U.S. Government Printing Office, Washington, D.C., 1969.

U.S. International Trade Commission, Synthetic Organic Chemicals, United States Production and Sales, 1975. USITC Publication 804, U.S. Government Printing Office, Washington, D.C., 1977.

APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN RATS TREATED WITH 2,3,5,6-TETRACHLORO-4-NITROANISOLE

TABLE A1
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS TREATED WITH
2,3,5,6-TETRACHLORO-4-NITROANISOLE

	CONTROL (UNTR) 01-0220	LOW DOSE 01-0270	HIGH DOSE 2 01-S275	HIGH DOSE 1 01-0275
ANIMALS INITIALLY IN STUDY	50	49	25	@50
ANIMALS NECROPSIED	48	49	25	23
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	48	49	25	23
INTEGUMENTARY SYSTEM				
*SKIN	(48)	(49)	(25)	(23)
SQUAMOUS CELL CARCINOMA		1 (2%)		
*SUBCUT TISSUE	(48)	(49)	(25)	(23)
SQUAMOUS CELL CARCINOMA			1 (4%)	
FIBROMA	3 (6%)	2 (4%)		
FIBROSARCOMA	2 (4%)			
RESPIRATORY SYSTEM				
*NASAL TURBINATE	(48)	(49)	(25)	(23)
SQUAMOUS CELL CARCINOMA			1 (4%)	
#LUNG	(48)	(49)	(25)	(23)
NEOPLASM, NOS		1 (2%)		
ALVEOLAR/BRONCHIOLAR ADENOMA	3 (6%)		1 (4%)	
FIBROSARCOMA, METASTATIC	1 (2%)			
HEMATOPOIETIC SYSTEM				
*MULTIPLE ORGANS	(48)	(49)	(25)	(23)
MALIGNANT LYMPHOMA, NOS	1 (2%)			
MALIG.LYMPHOMA, UNDIFFER-TYPE			1 (4%)	1 (4%)
LEUKEMIA,NOS		1 (2%)		
UNDIFFERENTIATED LEUKEMIA	2 (4%)			
MYELOMONOCYTIC LEUKEMIA		6 (12%)	2 (8%)	4 (17%)
*SPLEEN	(48)	(49)	(25)	(23)
MYELOMONOCYTIC LEUKEMIA	4 (8%)			
#LIVER	(48)	(49)	(25)	(23)
MALIG.LYMPHOMA, UNDIFFER-TYPE				1 (4%)

\# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
** EXCLUDES PARTIALLY AUTOLYZED ANIMALS
@ 50 ANIMALS WERE INITIALLY IN THE STUDY, BUT 26 WERE FOUND TO BE FEMALES IN A MALE GROUP.

TABLE A1 (CONTINUED)

	CONTROL (UNTR) 01-0220	LOW DOSE 01-0270	HIGH DOSE 2 01-S275	HIGH DOSE 1 01-0275
CIRCULATORY SYSTEM				
NONE				
DIGESTIVE SYSTEM				
#LIVER	(48)	(49)	(25)	(23)
NEOPLASTIC NODULE		3 (6%)	1 (4%)	
HEPATOCELLULAR CARCINOMA		2 (4%)		3 (13%)
#STOMACH	(48)	(48)	(25)	(23)
FIBROSARCOMA				1 (4%)
#DUODENUM	(46)	(49)	(24)	(23)
ISLET-CELL CARCINOMA, METASTATIC		1 (2%)		
URINARY SYSTEM				
NONE				
ENDOCRINE SYSTEM				
#PITUITARY	(45)	(43)	(21)	(20)
NEOPLASM, NOS		1 (2%)		
ADENOMA, NOS	10 (22%)	9 (21%)	4 (19%)	4 (20%)
#ADRENAL	(46)	(49)	(25)	(22)
CORTICAL ADENOMA		1 (2%)		
PHEOCHROMOCYTOMA	4 (9%)	4 (8%)	3 (12%)	4 (18%)
#THYROID	(43)	(45)	(22)	(22)
CARCINOMA,NOS		1 (2%)		
FOLLICULAR-CELL CARCINOMA			1 (5%)	1 (5%)
C-CELL ADENOMA	3 (7%)	1 (2%)		1 (5%)
C-CELL CARCINOMA	2 (5%)	1 (2%)		1 (5%)
#PARATHYROID	(25)	(21)	(7)	(3)
ADENOMA, NOS	1 (4%)			
#PANCREATIC ISLETS	(44)	(46)	(25)	(21)
ISLET-CELL ADENOMA		1 (2%)	1 (4%)	2 (10%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A1 (CONTINUED)

	CONTROL (UNTR) 01-0220	LOW DOSE 01-0270	HIGH DOSE [2] 01-S275	HIGH DOSE [1] 01-0275
ISLET-CELL CARCINOMA		1 (2%)		
REPRODUCTIVE SYSTEM				
*MAMMARY GLAND	(48)	(49)	(25)	(23)
FIBROADENOMA			1 (4%)	
#TESTIS	(48)	(49)	(24)	(23)
INTERSTITIAL-CELL TUMOR	37 (77%)	46 (94%)	19 (79%)	22 (96%)
NERVOUS SYSTEM				
#BRAIN	(46)	(48)	(24)	(22)
OSTEOSARCOMA, METASTATIC	1 (2%)			
SPECIAL SENSE ORGANS				
NONE				
MUSCULOSKELETAL SYSTEM				
*SKULL	(48)	(49)	(25)	(23)
OSTEOSARCOMA	1 (2%)			
BODY CAVITIES				
*BODY CAVITIES	(48)	(49)	(25)	(23)
MESOTHELIOMA, NOS	1 (2%)	1 (2%)	1 (4%)	
ALL OTHER SYSTEMS				
NONE				

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A1 (CONCLUDED)

	CONTROL(UNTR) 01-0220	LOW DOSE 01-0270	HIGH DOSE [2] 01-S275	HIGH DOSE [1] 01-0275
ANIMAL DISPOSITION SUMMARY				
ANIMALS INITIALLY IN STUDY	50	49	25	50
NATURAL DEATH@	8	5	5	3
MORIBUND SACRIFICE	5	3	4	
SCHEDULED SACRIFICE	5			
ACCIDENTALLY KILLED				
TERMINAL SACRIFICE	32	42	16	21
ANIMAL MISSING				
DELETED ANIMAL(WRONG SEX)				26
@ INCLUDES AUTOLYZED ANIMALS				
TUMOR SUMMARY				
TOTAL ANIMALS WITH PRIMARY TUMORS*	42	48	20	23
TOTAL PRIMARY TUMORS	74	83	37	45
TOTAL ANIMALS WITH BENIGN TUMORS	40	47	19	22
TOTAL BENIGN TUMORS	61	64	29	33
TOTAL ANIMALS WITH MALIGNANT TUMORS	11	13	6	10
TOTAL MALIGNANT TUMORS	12	13	6	12
TOTAL ANIMALS WITH SECONDARY TUMORS#	2	1		
TOTAL SECONDARY TUMORS	2	1		
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT	1	5	2	
TOTAL UNCERTAIN TUMORS	1	6	2	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC				
TOTAL UNCERTAIN TUMORS				

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS
\# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

TABLE A2
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS TREATED WITH
2,3,5,6-TETRACHLORO-4-NITROANISOLE

	CONTROL (UNTR) 02-0220	LOW DOSE 02-0270	HIGH DOSE 02-0275
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS MISSING			1
ANIMALS NECROPSIED	50	50	45
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	50	50	45
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(50)	(50)	(45)
BASAL-CELL TUMOR			1 (2%)
SARCOMA, NOS	1 (2%)		
FIBROSARCOMA		1 (2%)	1 (2%)
FIBROADENOMA		4 (8%)	
RESPIRATORY SYSTEM			
*NASAL CAVITY	(50)	(50)	(45)
PAPILLOMA, NOS		1 (2%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(50)	(50)	(45)
MALIGNANT LYMPHOMA, NOS	2 (4%)		
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE			1 (2%)
MYELOMONOCYTIC LEUKEMIA		6 (12%)	6 (13%)
#SPLEEN	(50)	(50)	(44)
MYELOMONOCYTIC LEUKEMIA	2 (4%)	1 (2%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(50)	(50)	(45)
NEOPLASTIC NODULE		1 (2%)	3 (7%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
** EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE A2 (CONTINUED)

	CONTROL(UNTR) 02-0220	LOW DOSE 02-0270	HIGH DOSE 02-0275
HEPATOCELLULAR CARCINOMA			1 (2%)
HEMANGIOMA			1 (2%)
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(40)	(48)	(40)
CARCINOMA,NOS		1 (2%)	1 (3%)
ADENOMA, NOS	16 (40%)	15 (31%)	14 (35%)
CHROMOPHOBE ADENOMA	1 (3%)		
#ADRENAL	(48)	(50)	(44)
CORTICAL ADENOMA		1 (2%)	1 (2%)
PHEOCHROMOCYTOMA	6 (13%)	1 (2%)	
#THYROID	(43)	(47)	(37)
NEOPLASM, NOS			1 (3%)
C-CELL ADENOMA		3 (6%)	
C-CELL CARCINOMA		1 (2%)	1 (3%)
#PANCREATIC ISLETS	(46)	(50)	(42)
ISLET-CELL ADENOMA		1 (2%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(50)	(50)	(45)
ADENOCARCINOMA, NOS	1 (2%)		
FIBROADENOMA	6 (12%)		1 (2%)
*CLITORAL GLAND	(50)	(50)	(45)
CARCINOMA,NOS		1 (2%)	
KERATOACANTHOMA			1 (2%)
#UTERUS	(48)	(50)	(45)
ADENOCARCINOMA, NOS	1 (2%)		
FIBROMA	2 (4%)		
LEIOMYOMA			1 (2%)
LEIOMYOSARCOMA			1 (2%)
ENDOMETRIAL STROMAL POLYP	2 (4%)	9 (18%)	8 (18%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A2 (CONTINUED)

	CONTROL(UNTR) 02-0220	LOW DOSE 02-0270	HIGH DOSE 02-0275
ENDOMETRIAL STROMAL SARCOMA	2 (4%)		
NERVOUS SYSTEM			
*BRAIN	(50)	(50)	(45)
CARCINOMA, NOS, INVASIVE		1 (2%)	1 (2%)
ASTROCYTOMA			1 (2%)
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*ABDOMINAL CAVITY	(50)	(50)	(45)
LEIOMYOSARCOMA		1 (2%)	
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH@	7	4	11
MORIBUND SACRIFICE	2	3	2
SCHEDULED SACRIFICE	5		
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	36	43	36
ANIMAL MISSING			1

@ INCLUDES AUTOLYZED ANIMALS

* NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A2 (CONCLUDED)

	CONTROL(UNTR) 02-0220	LOW DOSE 02-0270	HIGH DOSE 02-0275
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	27	34	28
TOTAL PRIMARY TUMORS	42	48	45
TOTAL ANIMALS WITH BENIGN TUMORS	23	30	20
TOTAL BENIGN TUMORS	33	35	28
TOTAL ANIMALS WITH MALIGNANT TUMORS	9	12	11
TOTAL MALIGNANT TUMORS	9	12	13
TOTAL ANIMALS WITH SECONDARY TUMORS#		1	1
TOTAL SECONDARY TUMORS		1	1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT		1	4
TOTAL UNCERTAIN TUMORS		1	4
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS
# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

APPENDIX B

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MICE TREATED WITH 2,3,5,6-TETRACHLORO-4-NITROANISOLE

TABLE B1
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE TREATED WITH
2,3,5,6-TETRACHLORO-4-NITROANISOLE

	CONTROL (UNTR) 05-0360	LOW DOSE 05-0271	HIGH DOSE 05-0276
ANIMALS INITIALLY IN STUDY	55	55	55
ANIMALS MISSING			1
ANIMALS NECROPSIED	55	55	53
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	55	54	52
INTEGUMENTARY SYSTEM			
*SKIN	(55)	(55)	(53)
SQUAMOUS CELL PAPILLOMA	1 (2%)		
*SUBCUT TISSUE	(55)	(55)	(53)
FIBROMA	2 (4%)		
FIBROSARCOMA	1 (2%)	1 (2%)	
LEIOMYOSARCOMA		2 (4%)	2 (4%)
HEMANGIOMA		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(54)	(54)	(51)
HEPATOCELLULAR CARCINOMA, METAST	4 (7%)		
ALVEOLAR/BRONCHIOLAR ADENOMA	6 (11%)	4 (7%)	4 (8%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	6 (11%)	13 (24%)	9 (18%)
CORTICAL CARCINOMA, METASTATIC		1 (2%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(55)	(55)	(53)
MALIGNANT LYMPHOMA, NOS		3 (5%)	
MALIG.LYMPHOMA, UNDIFFER-TYPE		1 (2%)	3 (6%)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		2 (4%)	1 (2%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (2%)		1 (2%)
MALIGNANT LYMPHOMA, MIXED TYPE	2 (4%)		3 (6%)
GRANULOCYTIC LEUKEMIA		1 (2%)	
#SPLEEN	(51)	(53)	(50)
NEOPLASM, NOS			1 (2%)
HEMANGIOMA	1 (2%)		
HEMANGIOSARCOMA			1 (2%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
** EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE B1 (CONTINUED)

	CONTROL(UNTR) 05-0360	LOW DOSE 05-0271	HIGH DOSE 05-0276
#MEDIASTINAL L.NODE	(48)	(50)	(47)
ALVEOLAR/BRONCHIOLAR CA, METASTA		1 (2%)	
#MESENTERIC L. NODE	(48)	(50)	(47)
HEPATOCELLULAR CARCINOMA, METAST	1 (2%)		
HEMANGIOSARCOMA		1 (2%)	
MALIG.LYMPHOMA, UNDIFFER-TYPE			1 (2%)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		1 (2%)	1 (2%)
MALIGNANT LYMPHOMA, MIXED TYPE			1 (2%)
#AXILLARY LYMPH NODE	(48)	(50)	(47)
LEIOMYOSARCOMA, METASTATIC			1 (2%)
#DUODENUM	(50)	(52)	(50)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		1 (2%)	
#JEJUNUM	(50)	(52)	(50)
MALIG.LYMPHOMA, UNDIFFER-TYPE			1 (2%)
MALIGNANT LYMPHOMA, MIXED TYPE	1 (2%)		
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(54)	(52)	(52)
HEPATOCELLULAR ADENOMA	4 (7%)	3 (6%)	2 (4%)
HEPATOCELLULAR CARCINOMA	24 (44%)	13 (25%)	9 (17%)
MIXED HEPATO/CHOLANGIO CARCINOMA		2 (4%)	2 (4%)
ANGIOSARCOMA		2 (4%)	3 (6%)
#STOMACH	(51)	(52)	(50)
SQUAMOUS CELL CARCINOMA		1 (2%)	
#DUODENUM	(50)	(52)	(50)
ADENOMATOUS POLYP, NOS		1 (2%)	
URINARY SYSTEM			
#KIDNEY/PELVIS	(54)	(53)	(52)
TRANSITIONAL-CELL PAPILLOMA	1 (2%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B1 (CONTINUED)

	CONTROL (UNTR) 05-0360	LOW DOSE 05-0271	HIGH DOSE 05-0276
ENDOCRINE SYSTEM			
*ADRENAL	(50)	(52)	(49)
CORTICAL ADENOMA	1 (2%)		
CORTICAL CARCINOMA		1 (2%)	
*ADRENAL/CAPSULE	(50)	(52)	(49)
ADENOMA, NOS	5 (10%)		
*THYROID	(48)	(49)	(44)
FOLLICULAR-CELL ADENOMA		1 (2%)	
FOLLICULAR-CELL CARCINOMA		2 (4%)	
*PANCREATIC ISLETS	(49)	(53)	(49)
ISLET-CELL ADENOMA	2 (4%)		
REPRODUCTIVE SYSTEM			
*PREPUTIAL GLAND	(55)	(55)	(53)
HEMANGIOSARCOMA			1 (2%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(55)	(55)	(53)
CYSTADENOMA, NOS	1 (2%)		
*EXTERNAL EAR	(55)	(55)	(53)
FIBROUS HISTIOCYTOMA			1 (2%)
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			

* NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B1 (CONCLUDED)

	CONTROL (UNTR) 05-0360	LOW DOSE 05-0271	HIGH DOSE 05-0276
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(55)	(55)	(53)
NEOPLASM, NOS			2 (4%)
SARCOMA, NOS			1 (2%)
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	55	55	55
NATURAL DEATH@	9	11	11
MORIBUND SACRIFICE	2	4	1
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			2
TERMINAL SACRIFICE	44	40	40
ANIMAL MISSING			1
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	43	38	36
TOTAL PRIMARY TUMORS	59	57	50
TOTAL ANIMALS WITH BENIGN TUMORS	22	8	6
TOTAL BENIGN TUMORS	24	10	7
TOTAL ANIMALS WITH MALIGNANT TUMORS	29	34	32
TOTAL MALIGNANT TUMORS	35	47	40
TOTAL ANIMALS WITH SECONDARY TUMORS#	4	2	1
TOTAL SECONDARY TUMORS	5	2	1
TOTAL ANIMALS WITH TUMORS UNCERTAIN- BENIGN OR MALIGNANT			3
TOTAL UNCERTAIN TUMORS			3
TOTAL ANIMALS WITH TUMORS UNCERTAIN- PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS
\# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

TABLE B2
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE TREATED WITH
2,3,5,6-TETRACHLORO-4-NITROANISOLE

	CONTROL (UNTR) 06-0360	LOW DOSE 06-0271	HIGH DOSE 06-0276
ANIMALS INITIALLY IN STUDY	55	55	55
ANIMALS MISSING		1	1
ANIMALS NECROPSIED	55	54	52
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	55	54	52
INTEGUMENTARY SYSTEM			
*SKIN	(55)	(54)	(52)
SQUAMOUS CELL CARCINOMA		1 (2%)	
*SUBCUT TISSUE	(55)	(54)	(52)
FIBROSARCOMA		1 (2%)	1 (2%)
HEMANGIOMA	1 (2%)		
RESPIRATORY SYSTEM			
*LUNG	(55)	(52)	(52)
HEPATOCELLULAR CARCINOMA, METAST	2 (4%)		1 (2%)
MIXED HEPATO/CHOLANGIOCA, METAST		1 (2%)	
ALVEOLAR/BRONCHIOLAR ADENOMA	3 (5%)	3 (6%)	2 (4%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	1 (2%)	5 (10%)	2 (4%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(55)	(54)	(52)
MALIGNANT LYMPHOMA, NOS	1 (2%)		
MALIG.LYMPHOMA, UNDIFFER-TYPE		2 (4%)	3 (6%)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	2 (4%)	11 (20%)	10 (19%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (2%)	2 (4%)	
MALIGNANT LYMPHOMA, MIXED TYPE	6 (11%)	2 (4%)	5 (10%)
LYMPHOCYTIC LEUKEMIA	4 (7%)		
*SPLEEN	(53)	(52)	(51)
HEMANGIOSARCOMA	2 (4%)		1 (2%)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		3 (6%)	
MALIGNANT LYMPHOMA, MIXED TYPE	1 (2%)	1 (2%)	1 (2%)
*MEDIASTINAL L.NODE	(47)	(50)	(46)
MIXED HEPATO/CHOLANGIOCA, METAST		1 (2%)	

* NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
** EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE B2 (CONTINUED)

	CONTROL(UNTR) 06-0360	LOW DOSE 06-0271	HIGH DOSE 06-0276
#MESENTERIC L. NODE	(47)	(50)	(46)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		1 (2%)	2 (4%)
MALIGNANT LYMPHOMA, MIXED TYPE	2 (4%)	1 (2%)	
#RENAL LYMPH NODE	(47)	(50)	(46)
SARCOMA, NOS			1 (2%)
#LIVER	(54)	(53)	(52)
MALIGNANT LYMPHOMA, NOS	1 (2%)		1 (2%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		2 (4%)	
#ILEUM	(52)	(53)	(51)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE			1 (2%)
#COLON	(48)	(51)	(49)
MALIG.LYMPHOMA, UNDIFFER-TYPE			1 (2%)
#THYMUS	(35)	(39)	(38)
ALVEOLAR/BRONCHIOLAR CA, METASTA		1 (3%)	
THYMOMA	1 (3%)		
MALIGNANT LYMPHOMA, MIXED TYPE			1 (3%)
CIRCULATORY SYSTEM			
#HEART	(55)	(53)	(52)
ALVEOLAR/BRONCHIOLAR CA, METASTA		1 (2%)	
HEMANGIOMA	1 (2%)		
DIGESTIVE SYSTEM			
#LIVER	(54)	(53)	(52)
HEPATOCELLULAR ADENOMA	4 (7%)	2 (4%)	2 (4%)
HEPATOCELLULAR CARCINOMA	7 (13%)	1 (2%)	3 (6%)
MIXED HEPATO/CHOLANGIO CARCINOMA		1 (2%)	
HEMANGIOMA	1 (2%)		
ANGIOSARCOMA			1 (2%)
#PANCREAS	(49)	(51)	(50)
MIXED HEPATO/CHOLANGIOCA, METAST		1 (2%)	
#STOMACH	(53)	(51)	(51)
PAPILLOMA, NOS			1 (2%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B2 (CONTINUED)

	CONTROL (UNTR) 06-0360	LOW DOSE 06-0271	HIGH DOSE 06-0276
SQUAMOUS CELL PAPILLOMA	2 (4%)		
MIXED HEPATO/CHOLANGIOCA, METAST		1 (2%)	
#DUODENUM	(52)	(53)	(51)
ADENOMATOUS POLYP, NOS			1 (2%)
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(42)	(47)	(41)
ADENOMA, NOS		2 (4%)	3 (7%)
CHROMOPHOBE ADENOMA	2 (5%)		
BASOPHIL ADENOMA	1 (2%)		
SARCOMA, NOS, METASTATIC		1 (2%)	
#ADRENAL	(50)	(53)	(49)
PHEOCHROMOCYTOMA	1 (2%)		
#THYROID	(48)	(39)	(48)
FOLLICULAR-CELL ADENOMA	1 (2%)		1 (2%)
FOLLICULAR-CELL CARCINOMA			1 (2%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(55)	(54)	(52)
ADENOCARCINOMA, NOS		1 (2%)	1 (2%)
ACINAR-CELL CARCINOMA	1 (2%)		
FIBROADENOMA	1 (2%)		
#UTERUS	(54)	(51)	(52)
NEOPLASM, NOS, MALIGNANT	1 (2%)		
LEIOMYOMA		1 (2%)	
ENDOMETRIAL STROMAL POLYP		2 (4%)	
#OVARY	(50)	(49)	(49)
MIXED HEPATO/CHOLANGIOCA, METAST		1 (2%)	
PAPILLARY CYSTADENOMA, NOS			1 (2%)
CHORIOCARCINOMA			1 (2%)
HEMANGIOSARCOMA			1 (2%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B2 (CONTINUED)

	CONTROL(UNTR) 06-0360	LOW DOSE 06-0271	HIGH DOSE 06-0276
NERVOUS SYSTEM			
*BRAIN	(55)	(52)	(52)
SARCOMA, NOS, METASTATIC		1 (2%)	
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(55)	(54)	(52)
PAPILLARY ADENOMA			1 (2%)
PAPILLARY CYSTADENOMA, NOS		1 (2%)	1 (2%)
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*BODY CAVITIES	(55)	(54)	(52)
MESOTHELIOMA, NOS	1 (2%)		
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(55)	(54)	(52)
SARCOMA, NOS			1 (2%)
HEAD			
SARCOMA, NOS		1	
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	55	55	55
NATURAL DEATH@	7	11	10
MORIBUND SACRIFICE	4	5	1
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	44	38	43
ANIMAL MISSING		1	1

@ INCLUDES AUTOLYZED ANIMALS

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B2 (CONCLUDED)

	CONTROL(UNTR) 06-0360	LOW DOSE 06-0271	HIGH DOSE 06-0276
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	34	37	38
TOTAL PRIMARY TUMORS	50	47	52
TOTAL ANIMALS WITH BENIGN TUMORS	15	9	12
TOTAL BENIGN TUMORS	19	11	13
TOTAL ANIMALS WITH MALIGNANT TUMORS	27	34	32
TOTAL MALIGNANT TUMORS	30	36	39
TOTAL ANIMALS WITH SECONDARY TUMORS#	2	3	1
TOTAL SECONDARY TUMORS	2	9	1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT	1		
TOTAL UNCERTAIN TUMORS	1		
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS
\# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

APPENDIX C

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN RATS TREATED WITH 2,3,5,6-TETRACHLORO-4-NITROANISOLE

TABLE C1
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS TREATED WITH
2,3,5,6-TETRACHLORO-4-NITROANISOLE

	CONTROL (UNTR) 01-0220	LOW DOSE 01-0270	HIGH DOSE 2 01-S275	HIGH DOSE 1 01-0275
ANIMALS INITIALLY IN STUDY	50	49	25	@50
ANIMALS NECROPSIED	48	49	25	23
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	48	49	25	23
INTEGUMENTARY SYSTEM				
*SKIN	(48)	(49)	(25)	(23)
EPIDERMAL INCLUSION CYST	1 (2%)	1 (2%)		
ULCER, ACUTE		1 (2%)		
DEGENERATION, NOS		1 (2%)		
NECROSIS, NOS		1 (2%)		
*SUBCUT TISSUE	(48)	(49)	(25)	(23)
ABSCESS, NOS	3 (6%)	1 (2%)		
RESPIRATORY SYSTEM				
*NASAL TURBINATE	(48)	(49)	(25)	(23)
INFLAMMATION, SUPPURATIVE	1 (2%)			
#LUNG/BRONCHUS	(48)	(49)	(25)	(23)
BRONCHIECTASIS		1 (2%)		
#LUNG	(48)	(49)	(25)	(23)
EDEMA, NOS				1 (4%)
INFLAMMATION, FOCAL GRANULOMATOU		2 (4%)		1 (4%)
PERIVASCULAR CUFFING		1 (2%)		
HYPERPLASIA, ADENOMATOUS		2 (4%)	1 (4%)	
HISTIOCYTOSIS		1 (2%)		
HYPERPLASIA, LYMPHOID			1 (4%)	
HEMATOPOIETIC SYSTEM				
#BONE MARROW	(46)	(47)	(25)	(22)
MYELOFIBROSIS				1 (5%)
#SPLEEN	(48)	(49)	(25)	(23)
NECROSIS, FOCAL		1 (2%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
** EXCLUDES PARTIALLY AUTOLYZED ANIMALS
@ 50 ANIMALS WERE INITIALLY IN THE STUDY, BUT 26 WERE FOUND TO BE FEMALES IN A MALE GROUP.

TABLE C1 (CONTINUED)

	CONTROL (UNTR) 01-0220	LOW DOSE 01-0270	HIGH DOSE 2 01-S275	HIGH DOSE 1 01-0275
INFARCT, NOS	1 (2%)			
LEUKEMOID REACTION		1 (2%)		
#MANDIBULAR L. NODE	(43)	(46)	(23)	(19)
PLASMA-CELL INFILTRATE		2 (4%)		
HISTIOCYTOSIS		1 (2%)		
PLASMACYTOSIS		2 (4%)		
#CERVICAL LYMPH NODE	(43)	(46)	(23)	(19)
PLASMA-CELL INFILTRATE		1 (2%)		
HISTIOCYTOSIS		1 (2%)		
#RENAL LYMPH NODE	(43)	(46)	(23)	(19)
PLASMACYTOSIS				1 (5%)
#THYMUS	(32)	(34)	(19)	(16)
CYST, NOS				1 (6%)
ATROPHY, NOS			1 (5%)	
HYPERPLASIA, NOS	1 (3%)			
CIRCULATORY SYSTEM				
#HEART	(48)	(49)	(25)	(23)
THROMBOSIS, NOS				1 (4%)
FIBROSIS		1 (2%)		
FIBROSIS, FOCAL		19 (39%)	5 (20%)	14 (61%)
DIGESTIVE SYSTEM				
#LIVER	(48)	(49)	(25)	(23)
THROMBOSIS, NOS				1 (4%)
HEMORRHAGE			1 (4%)	
LYMPHOCYTIC INFLAMMATORY INFILTR				1 (4%)
NECROSIS, NOS				1 (4%)
NECROSIS, FOCAL		1 (2%)	1 (4%)	
METAMORPHOSIS FATTY	2 (4%)			
LIPOIDOSIS				1 (4%)
CYTOPLASMIC VACUOLIZATION			1 (4%)	1 (4%)
BASOPHILIC CYTO CHANGE		4 (8%)		3 (13%)
CLEAR-CELL CHANGE		3 (6%)		2 (9%)
HYPERPLASIA, FOCAL	3 (6%)			
ANGIECTASIS		2 (4%)		1 (4%)
#LIVER/HEPATOCYTES	(48)	(49)	(25)	(23)
HYPERTROPHY, FOCAL		1 (2%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C1 (CONTINUED)

	CONTROL (UNTR) 01-0220	LOW DOSE 01-0270	HIGH DOSE 2 01-S275	HIGH DOSE 1 01-0275
*BILE DUCT	(48)	(49)	(25)	(23)
HYPERPLASIA, NOS		6 (12%)	1 (4%)	9 (39%)
#PANCREAS	(44)	(46)	(25)	(21)
INFLAMMATION, NOS	2 (5%)			
ATROPHY, FOCAL			1 (4%)	
#PANCREATIC ACINUS	(44)	(46)	(25)	(21)
ATROPHY, NOS		4 (9%)		1 (5%)
ATROPHY, FOCAL		8 (17%)	2 (8%)	3 (14%)
#STOMACH	(48)	(48)	(25)	(23)
EDEMA, NOS			1 (4%)	
INFLAMMATION, ACUTE/CHRONIC			1 (4%)	
INFLAMMATION, CHRONIC				1 (4%)
HYPERPLASIA, PSEUDOEPITHELIOMATO				1 (4%)
HYPERKERATOSIS			1 (4%)	
ACANTHOSIS			1 (4%)	
#GASTRIC MUCOSA	(48)	(48)	(25)	(23)
HYPERPLASIA, DIFFUSE			1 (4%)	
#JEJUNUM	(46)	(49)	(24)	(23)
PARASITISM				1 (4%)
#ILEUM	(46)	(49)	(24)	(23)
INFLAMMATION, FOCAL			1 (4%)	
#COLON	(42)	(49)	(23)	(23)
LYMPHOCYTIC INFLAMMATORY INFILTR				1 (4%)
PARASITISM		6 (12%)	5 (22%)	2 (9%)
URINARY SYSTEM				
#KIDNEY	(48)	(49)	(25)	(23)
EMBOLUS, SEPTIC				1 (4%)
INFLAMMATION, FOCAL				1 (4%)
INFLAMMATION, SUPPURATIVE				1 (4%)
NEPHROPATHY	35 (73%)	20 (41%)	11 (44%)	11 (48%)
METAPLASIA, OSSEOUS		1 (2%)		
#KIDNEY/TUBULE	(48)	(49)	(25)	(23)
PIGMENTATION, NOS		1 (2%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C1 (CONTINUED)

	CONTROL(UNTR) 01-0220	LOW DOSE 01-0270	HIGH DOSE [2] 01-S275	HIGH DOSE [1] 01-0275
ENDOCRINE SYSTEM				
#PITUITARY	(45)	(43)	(21)	(20)
HYPERPLASIA, FOCAL	1 (2%)	4 (9%)		
#ADRENAL	(46)	(49)	(25)	(22)
NECROSIS, FOCAL		1 (2%)		
ANGIECTASIS		1 (2%)		
HEMATOPOIESIS			1 (4%)	
#ADRENAL CORTEX	(46)	(49)	(25)	(22)
HEMORRHAGE			1 (4%)	
NECROSIS, NOS			1 (4%)	
LIPOIDOSIS			1 (4%)	
ANGIECTASIS			1 (4%)	
#ADRENAL MEDULLA	(46)	(49)	(25)	(22)
HYPERPLASIA, NOS	1 (2%)			
HYPERPLASIA, FOCAL	3 (7%)	3 (6%)	1 (4%)	
#THYROID	(43)	(45)	(22)	(22)
HYPERPLASIA, C-CELL		1 (2%)		
#PANCREATIC ISLETS	(44)	(46)	(25)	(21)
HYPERPLASIA, NOS	1 (2%)	1 (2%)		
REPRODUCTIVE SYSTEM				
*MAMMARY GLAND	(48)	(49)	(25)	(23)
DISPLACEMENT, NOS		1 (2%)		
LACTATION		1 (2%)	1 (4%)	
#PROSTATE	(45)	(49)	(24)	(23)
INFLAMMATION, SUPPURATIVE			1 (4%)	1 (4%)
INFLAMMATION, ACUTE				2 (9%)
INFLAMMATION, ACUTE/CHRONIC			1 (4%)	3 (13%)
*SEMINAL VESICLE	(48)	(49)	(25)	(23)
INFLAMMATION, SUPPURATIVE				1 (4%)
#TESTIS	(48)	(49)	(24)	(23)
MINERALIZATION	1 (2%)			
ATROPHY, NOS	4 (8%)	2 (4%)	3 (13%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C1 (CONTINUED)

	CONTROL(UNTR) 01-0220	LOW DOSE 01-0270	HIGH DOSE 2 01-S275	HIGH DOSE 1 01-0275
HYPERPLASIA, INTERSTITIAL CELL	3 (6%)		1 (4%)	
*EPIDIDYMIS	(48)	(49)	(25)	(23)
DILATATION/DUCTS		1 (2%)		
ABSCESS, NOS	1 (2%)			
NERVOUS SYSTEM				
#BRAIN	(46)	(48)	(24)	(22)
EMBOLUS, SEPTIC				1 (5%)
HEMORRHAGE		1 (2%)		
FIBROSIS				1 (5%)
NECROSIS, HEMORRHAGIC		1 (2%)		
SPECIAL SENSE ORGANS				
*EYE	(48)	(49)	(25)	(23)
CATARACT	1 (2%)			1 (4%)
*EYE/RETINA	(48)	(49)	(25)	(23)
ATROPHY, NOS	2 (4%)			
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
*MESENTERY	(48)	(49)	(25)	(23)
PERIAPTERITIS		1 (2%)		
ALL OTHER SYSTEMS				
NONE				
SPECIAL MORPHOLOGY SUMMARY				
NO LESION REPORTED			2	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C1 (CONCLUDED)

	CONTROL(UNTR) 01-0220	LOW DOSE 01-0270	HIGH DOSE [2] 01-S275	HIGH DOSE [1] 01-0275
AUTO/NECROPSY/HISTO PERF	1			
AUTO/NECROPSY/NO HISTO		1		
AUTOLYSIS/NO NECROPSY	2			1

* NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C2
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS TREATED WITH 2,3,5,6-TETRACHLORO-4-NITROANISOLE

	CONTROL (UNTR) 02-0220	LOW DOSE 02-0270	HIGH DOSE 02-0275
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS MISSING			1
ANIMALS NECROPSIED	50	50	45
ANIMALS EXAMINED HISTOPATHOLOGICALLY **	50	50	45
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(50)	(45)
EPIDERMAL INCLUSION CYST			1 (2%)
FIBROSIS, FOCAL			1 (2%)
POLYPOID HYPERPLASIA			1 (2%)
HISTIOCYTOSIS		1 (2%)	
RESPIRATORY SYSTEM			
*NASAL TURBINATE	(50)	(50)	(45)
INFLAMMATION, NOS	1 (2%)		
#LUNG/BRONCHUS	(50)	(50)	(44)
BRONCHIECTASIS		1 (2%)	
#LUNG	(50)	(50)	(44)
ATELECTASIS			1 (2%)
INFLAMMATION, NOS	1 (2%)		
LYMPHOCYTIC INFLAMMATORY INFILTR			1 (2%)
INFLAMMATION, INTERSTITIAL	1 (2%)		
BRONCHOPNEUMONIA SUPPURATIVE			1 (2%)
ABSCESS, NOS	2 (4%)		
PNEUMONIA, CHRONIC MURINE	1 (2%)		
GRANULOMA, NOS	1 (2%)		
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(48)	(47)	(42)
MYELOFIBROSIS			1 (2%)
#SPLEEN	(50)	(50)	(44)
HEMATOPOIESIS	7 (14%)	6 (12%)	3 (7%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
** EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE C2 (CONTINUED)

	CONTROL(UNTR) 02-0220	LOW DOSE 02-0270	HIGH DOSE 02-0275
#LUMBAR LYMPH NODE	(43)	(49)	(43)
HYPERPLASIA, NOS		1 (2%)	
#RENAL LYMPH NODE	(43)	(49)	(43)
SIDEROSIS		1 (2%)	
#THYMUS	(31)	(40)	(32)
HEMORRHAGE		1 (3%)	1 (3%)
ATROPHY, NOS		1 (3%)	
CIRCULATORY SYSTEM			
#HEART	(50)	(50)	(44)
FIBROSIS, FOCAL		1 (2%)	1 (2%)
#ENDOCARDIUM	(50)	(50)	(44)
INFLAMMATION, CHRONIC		1 (2%)	
DIGESTIVE SYSTEM			
#LIVER	(50)	(50)	(45)
CONGESTION, NOS		1 (2%)	
HEMORRHAGE			1 (2%)
INFLAMMATION, CHRONIC		1 (2%)	
INFLAMMATION, CHRONIC FOCAL			1 (2%)
INFLAMMATION, FOCAL GRANULOMATOU		1 (2%)	
CHOLANGIOFIBROSIS			1 (2%)
NECROSIS, NOS			1 (2%)
NECROSIS, FOCAL		1 (2%)	
METAMORPHOSIS FATTY	4 (8%)		
BASOPHILIC CYTO CHANGE		25 (50%)	16 (36%)
HYPERPLASIA, FOCAL	9 (18%)		
ANGIECTASIS		1 (2%)	
#LIVER/CENTRILOBULAR	(50)	(50)	(45)
NECROSIS, NOS			1 (2%)
#BILE DUCT	(50)	(50)	(45)
CYST, NOS			1 (2%)
HYPERPLASIA, NOS			1 (2%)
#PANCREATIC ACINUS	(46)	(50)	(42)
ATROPHY, NOS		1 (2%)	

* NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C2 (CONTINUED)

	CONTROL (UNTR) 02-0220	LOW DOSE 02-0270	HIGH DOSE 02-0275
ATROPHY, FOCAL			4 (10%)
#STOMACH	(49)	(50)	(44)
FIBROSIS, FOCAL			1 (2%)
HYPERPLASIA, PAPILLARY	1 (2%)		
#ILEUM	(47)	(50)	(44)
CALCIFICATION, METASTATIC			1 (2%)
#COLON	(40)	(47)	(42)
PARASITISM		6 (13%)	4 (10%)
URINARY SYSTEM			
#KIDNEY	(49)	(50)	(45)
GLOMERULONEPHRITIS, NOS	1 (2%)		
NEPHROPATHY	18 (37%)	3 (6%)	3 (7%)
#KIDNEY/TUBULE	(49)	(50)	(45)
PIGMENTATION, NOS		1 (2%)	
CYTOPLASMIC VACUOLIZATION			1 (2%)
#URINARY BLADDER	(47)	(49)	(45)
HEMORRHAGE			1 (2%)
HYPERPLASIA, EPITHELIAL			1 (2%)
ENDOCRINE SYSTEM			
#PITUITARY	(40)	(48)	(40)
MINERALIZATION	1 (3%)		1 (3%)
HEMORRHAGE			2 (5%)
SIDEROSIS			1 (3%)
HYPERPLASIA, FOCAL		1 (2%)	
#ADRENAL	(48)	(50)	(44)
ANGIECTASIS		1 (2%)	
#ADRENAL CORTEX	(48)	(50)	(44)
CYTOPLASMIC VACUOLIZATION		2 (4%)	
#THYROID	(43)	(47)	(37)
CYSTIC FOLLICLES		1 (2%)	1 (3%)
HYPERPLASIA, CYSTIC			1 (3%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C2 (CONTINUED)

	CONTROL(UNTR) 02-0220	LOW DOSE 02-0270	HIGH DOSE 02-0275
HYPERPLASIA, C-CELL	1 (2%)	1 (2%)	3 (8%)
HYPERPLASIA, FOLLICULAR-CELL			1 (3%)
HYPERPLASIA, LYMPHOID			1 (3%)
#PARATHYROID	(31)	(14)	(21)
HYPERPLASIA, NODULAR	1 (3%)		
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(50)	(50)	(45)
DISPLACEMENT, NOS		8 (16%)	6 (13%)
DILATATION/DUCTS		1 (2%)	2 (4%)
GALACTOCELE	2 (4%)		
FIBROSIS, DIFFUSE			1 (2%)
HYPERPLASIA, NOS	1 (2%)		
LACTATION		1 (2%)	
*ACINUS OF BREAST	(50)	(50)	(45)
DILATATION, NOS			1 (2%)
*CLITORAL GLAND	(50)	(50)	(45)
CYST, NOS			1 (2%)
INFLAMMATION, ACUTE			1 (2%)
HYPERPLASIA, CYSTIC		1 (2%)	1 (2%)
#UTERUS	(48)	(50)	(45)
DILATATION, NOS		6 (12%)	3 (7%)
HYDROMETRA		1 (2%)	
INFLAMMATION, SUPPURATIVE		1 (2%)	5 (11%)
INFLAMMATION, ACUTE		1 (2%)	
ABSCESS, NOS	1 (2%)		
ABSCESS, CHRONIC		1 (2%)	
NECROSIS, NOS	1 (2%)		
DECIDUA			1 (2%)
#CERVIX UTERI	(48)	(50)	(45)
HEMATOMA, NOS		1 (2%)	
#UTERUS/ENDOMETRIUM	(48)	(50)	(45)
CYST, NOS			2 (4%)
INFLAMMATION, NOS	1 (2%)		
HYPERPLASIA, NOS			1 (2%)
HYPERPLASIA, CYSTIC		1 (2%)	
#OVARY	(49)	(50)	(44)
CYST, NOS		4 (8%)	2 (5%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C2 (CONCLUDED)

	CONTROL (UNTR) 02-0220	LOW DOSE 02-0270	HIGH DOSE 02-0275
INFLAMMATION, NOS	1 (2%)		
INFLAMMATION, SUPPURATIVE			1 (2%)
INFLAMMATION, ACUTE			1 (2%)
INFLAMMATION, CHRONIC			2 (5%)
DEGENERATION, CYSTIC	2 (4%)		
NERVOUS SYSTEM			
#BRAIN	(50)	(50)	(45)
HEMORRHAGE			2 (4%)
NECROSIS, NOS			2 (4%)
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
DIAPHRAGM			
HERNIA, NOS		1	
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	11	1	3
ANIMAL MISSING/NO NECROPSY			1
AUTO/NECROPSY/HISTO PERF	1		1
AUTOLYSIS/NO NECROPSY			4

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

APPENDIX D

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MICE TREATED WITH 2,3,5,6-TETRACHLORO-4-NITROANISOLE

TABLE D1
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE TREATED WITH
2,3,5,6-TETRACHLORO-4-NITROANISOLE

	CONTROL (UNTR) 05-0360	LOW DOSE 05-0271	HIGH DOSE 05-0276
ANIMALS INITIALLY IN STUDY	55	55	55
ANIMALS MISSING			1
ANIMALS NECROPSIED	55	55	53
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	55	54	52
INTEGUMENTARY SYSTEM			
*SKIN	(55)	(55)	(53)
EPIDERMAL INCLUSION CYST	1 (2%)		
INFLAMMATION, GRANULOMATOUS		1 (2%)	
ACANTHOSIS		1 (2%)	
POLYP, INFLAMMATORY	1 (2%)		
RESPIRATORY SYSTEM			
*LUNG	(54)	(54)	(51)
EDEMA, NOS		1 (2%)	
HEMORRHAGE		1 (2%)	1 (2%)
HYPERPLASIA, ADENOMATOUS		1 (2%)	
HYPERPLASIA, ALVEOLAR EPITHELIUM		1 (2%)	
HEMATOPOIETIC SYSTEM			
*SPLEEN	(51)	(53)	(50)
INFLAMMATION, PYOGRANULOMATOUS		1 (2%)	
ATROPHY, NOS		1 (2%)	
PLASMACYTOSIS		1 (2%)	
HYPERPLASIA, LYMPHOID		1 (2%)	1 (2%)
HEMATOPOIESIS	2 (4%)	2 (4%)	6 (12%)
*PANCREATIC L.NODE	(48)	(50)	(47)
PLASMACYTOSIS		1 (2%)	
*AORTIC LYMPH NODE	(48)	(50)	(47)
THROMBOSIS, NOS		1 (2%)	
*LUMBAR LYMPH NODE	(48)	(50)	(47)
HEMORRHAGE		1 (2%)	

* NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
** EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE D1 (CONTINUED)

	CONTROL(UNTR) 05-0360	LOW DOSE 05-0271	HIGH DOSE 05-0276
*MESENTERIC L. NODE	(48)	(50)	(47)
CONGESTION, NOS	6 (13%)		
HEMORRHAGE		1 (2%)	
HYPERPLASIA, NOS	1 (2%)		
HISTIOCYTOSIS	1 (2%)	1 (2%)	1 (2%)
PLASMACYTOSIS		1 (2%)	
HYPERPLASIA, LYMPHOID		1 (2%)	2 (4%)
HEMATOPOIESIS		15 (30%)	10 (21%)
#RENAL LYMPH NODE	(48)	(50)	(47)
HEMORRHAGE		1 (2%)	
HYPERPLASIA, LYMPHOID		1 (2%)	
#THYMUS	(39)	(36)	(39)
ATROPHY, NOS			1 (3%)
CIRCULATORY SYSTEM			
#HEART	(55)	(54)	(52)
THROMBOSIS, NOS		1 (2%)	
FIBROSIS		1 (2%)	
FIBROSIS, DIFFUSE			1 (2%)
DIGESTIVE SYSTEM			
#LIVER	(54)	(52)	(52)
INFLAMMATION, GRANULOMATOUS			1 (2%)
INFARCT, NOS		2 (4%)	
CYTOPLASMIC VACUOLIZATION		4 (8%)	1 (2%)
BASOPHILIC CYTO CHANGE			1 (2%)
EOSINOPHILIC CYTO CHANGE		1 (2%)	
CLEAR-CELL CHANGE		3 (6%)	1 (2%)
HYPERPLASIA, NOS	1 (2%)		
#LIVER/CENTRILOBULAR	(54)	(52)	(52)
CYTOPLASMIC VACUOLIZATION		1 (2%)	
*BILE DUCT	(55)	(55)	(53)
CYST, NOS		1 (2%)	2 (4%)
#PANCREAS	(49)	(53)	(49)
DILATATION/DUCTS		1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D1 (CONTINUED)

	CONTROL(UNTR) 05-0360	LOW DOSE 05-0271	HIGH DOSE 05-0276
ATROPHY, NOS	1 (2%)		
ATROPHY, FOCAL		1 (2%)	
#STOMACH	(51)	(52)	(50)
ULCER, NOS			2 (4%)
LYMPHOCYTIC INFLAMMATORY INFILTR			1 (2%)
INFLAMMATION, ACUTE	1 (2%)		1 (2%)
ATYPIA, NOS	1 (2%)		
HYPERKERATOSIS		3 (6%)	5 (10%)
ACANTHOSIS		4 (8%)	5 (10%)
#JEJUNUM	(50)	(52)	(50)
DIVERTICULUM			1 (2%)
HYPERPLASIA, LYMPHOID			1 (2%)
#ILEUM	(50)	(52)	(50)
HYPERPLASIA, LYMPHOID		2 (4%)	2 (4%)
#COLON	(45)	(49)	(46)
PARASITISM		1 (2%)	4 (9%)
URINARY SYSTEM			
#KIDNEY	(54)	(53)	(52)
HYDRONEPHROSIS	1 (2%)		
PYELONEPHRITIS, ACUTE	1 (2%)		
PYELONEPHRITIS, CHRONIC	1 (2%)		
FIBROSIS, FOCAL		1 (2%)	
NEPHROPATHY		1 (2%)	
NECROSIS, CORTICAL		1 (2%)	
#URINARY BLADDER	(48)	(52)	(51)
CALCULUS, NOS	1 (2%)		
HYPERPLASIA, EPITHELIAL	1 (2%)		
ENDOCRINE SYSTEM			
#ADRENAL	(50)	(52)	(49)
NECROSIS, CORTICAL			1 (2%)
#PANCREATIC ISLETS	(49)	(53)	(49)
HYPERPLASIA, NOS	12 (24%)		

* NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D1 (CONTINUED)

	CONTROL(UNTR) 05-0360	LOW DOSE 05-0271	HIGH DOSE 05-0276
REPRODUCTIVE SYSTEM			
*PREPUTIAL GLAND	(55)	(55)	(53)
CALCULUS, NOS	1 (2%)		
DILATATION/DUCTS		1 (2%)	
CYST, NOS		1 (2%)	1 (2%)
INFLAMMATION, SUPPURATIVE		1 (2%)	
HYPERPLASIA, CYSTIC		1 (2%)	
#PROSTATE	(52)	(50)	(49)
INFLAMMATION, ACUTE	1 (2%)		
HYPERPLASIA, PAPILLARY		1 (2%)	
#TESTIS	(54)	(52)	(50)
ATROPHY, NOS		3 (6%)	1 (2%)
*EPIDIDYMIS	(55)	(55)	(53)
MULTINUCLEATE GIANT-CELL			1 (2%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*EYE	(55)	(55)	(53)
INFLAMMATION, ACUTE	1 (2%)		
CATARACT	1 (2%)		
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*ABDOMINAL CAVITY	(55)	(55)	(53)
NECROSIS, FAT	5 (9%)		
ALL OTHER SYSTEMS			
OMENTUM			
HEMATOMA, NOS	1		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D1 (CONCLUDED)

	CONTROL (UNTR) 05-0360	LOW DOSE 05-0271	HIGH DOSE 05-0276
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	8	5	4
ANIMAL MISSING/NO NECROPSY			1
AUTO/NECROPSY/HISTO PERF	1		
AUTO/NECROPSY/NO HISTO		1	1
AUTOLYSIS/NO NECROPSY			1

* NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D2
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE TREATED WITH 2,3,5,6-TETRACHLORO-4-NITROANISOLE

	CONTROL (UNTR) 06-0360	LOW DOSE 06-0271	HIGH DOSE 06-0276
ANIMALS INITIALLY IN STUDY	55	55	55
ANIMALS MISSING		1	1
ANIMALS NECROPSIED	55	54	52
ANIMALS EXAMINED HISTOPATHOLOGICALLY **	55	54	52
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(55)	(52)	(52)
ATELECTASIS	1 (2%)		
INFLAMMATION, SUPPURATIVE			1 (2%)
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(52)	(54)	(52)
MYELOFIBROSIS	31 (60%)	26 (48%)	18 (35%)
HYPERPLASIA, HEMATOPOIETIC	1 (2%)		
#SPLEEN	(53)	(52)	(51)
HYPERPLASIA, LYMPHOID		1 (2%)	1 (2%)
HEMATOPOIESIS	1 (2%)	7 (13%)	4 (8%)
#LUMBAR LYMPH NODE	(47)	(50)	(46)
INFLAMMATION, SUPPURATIVE		1 (2%)	
HYPERPLASIA, LYMPHOID		2 (4%)	1 (2%)
#MESENTERIC L. NODE	(47)	(50)	(46)
THROMBOSIS, NOS		1 (2%)	
CONGESTION, NOS	1 (2%)		
HYPERPLASIA, NOS	2 (4%)		
HYPERPLASIA, LYMPHOID		3 (6%)	1 (2%)
HEMATOPOIESIS		2 (4%)	
#RENAL LYMPH NODE	(47)	(50)	(46)
INFLAMMATION, ACUTE/CHRONIC		1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
** EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE D2 (CONTINUED)

	CONTROL(UNTR) 06-0360	LOW DOSE 06-0271	HIGH DOSE 06-0276
HYPERPLASIA, LYMPHOID		2 (4%)	
#AXILLARY LYMPH NODE	(47)	(50)	(46)
INFLAMMATION, SUPPURATIVE		1 (2%)	
#THYMUS	(35)	(39)	(38)
ATROPHY, NOS		1 (3%)	
HYPERPLASIA, LYMPHOID		1 (3%)	
CIRCULATORY SYSTEM			
#HEART	(55)	(53)	(52)
PERIARTERITIS	1 (2%)		
DEGENERATION, MUCOID		1 (2%)	
DIGESTIVE SYSTEM			
#LIVER	(54)	(53)	(52)
CYTOPLASMIC VACUOLIZATION		2 (4%)	
CLEAR-CELL CHANGE			1 (2%)
#PANCREAS	(49)	(51)	(50)
DILATATION/DUCTS	1 (2%)	1 (2%)	3 (6%)
INFLAMMATION, CHRONIC	2 (4%)		
ATROPHY, NOS	1 (2%)		3 (6%)
ATROPHY, FOCAL		1 (2%)	
ATROPHY, FATTY		1 (2%)	
#PANCREATIC ACINUS	(49)	(51)	(50)
ATROPHY, FATTY		1 (2%)	
#STOMACH	(53)	(51)	(51)
ULCER, NOS	1 (2%)	1 (2%)	
ABSCESS, NOS			1 (2%)
INFLAMMATION, CHRONIC		1 (2%)	
INFLAMMATION, CHRONIC FOCAL		1 (2%)	
EROSION	1 (2%)		
HYPERPLASIA, EPITHELIAL	2 (4%)		
HYPERPLASIA, PSEUDOEPITHELIOMATO			1 (2%)
HYPERKERATOSIS		3 (6%)	7 (14%)
ACANTHOSIS		4 (8%)	7 (14%)
URINARY SYSTEM			
#KIDNEY	(55)	(53)	(52)
PYELONEPHRITIS, CHRONIC	1 (2%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D2 (CONTINUED)

	CONTROL (UNTR) 06-0360	LOW DOSE 06-0271	HIGH DOSE 06-0276
INFLAMMATION, CHRONIC FOCAL		1 (2%)	
NEPHROPATHY		1 (2%)	
DEGENERATION, HYALINE		1 (2%)	
#URINARY BLADDER	(50)	(54)	(49)
LYMPHOCYTIC INFLAMMATORY INFILTR			1 (2%)
ULCER, ACUTE			1 (2%)
INFLAMMATION, CHRONIC	1 (2%)		
ENDOCRINE SYSTEM			
#PITUITARY	(42)	(47)	(41)
HYPERPLASIA, FOCAL		1 (2%)	
#ADRENAL	(50)	(53)	(49)
NECROSIS, NOS		1 (2%)	
ANGIECTASIS		1 (2%)	
#THYROID	(48)	(39)	(48)
FOLLICULAR CYST, NOS			1 (2%)
HYPERPLASIA, CYSTIC		1 (3%)	1 (2%)
#PANCREATIC ISLETS	(49)	(51)	(50)
HYPERPLASIA, NOS	3 (6%)		
REPRODUCTIVE SYSTEM			
#UTERUS	(54)	(51)	(52)
HYDROMETRA	3 (6%)		
DEGENERATION, HYALINE			2 (4%)
NECROSIS, FOCAL			1 (2%)
#UTERUS/ENDOMETRIUM	(54)	(51)	(52)
HYPERPLASIA, CYSTIC	15 (28%)	35 (69%)	32 (62%)
#OVARY	(50)	(49)	(49)
CYST, NOS	7 (14%)		1 (2%)
FOLLICULAR CYST, NOS		5 (10%)	3 (6%)
HEMORRHAGIC CYST	1 (2%)		4 (8%)
ABSCESS, NOS	1 (2%)		1 (2%)
NERVOUS SYSTEM			
#BRAIN/MENINGES	(55)	(52)	(52)
INFLAMMATION, NOS	1 (2%)		

\# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D2 (CONCLUDED)

	CONTROL (UNTR) 06-0360	LOW DOSE 06-0271	HIGH DOSE 06-0276
#BRAIN	(55)	(52)	(52)
HYDROCEPHALUS, NOS	2 (4%)		
DEGENERATION, NOS		1 (2%)	
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*ABDOMINAL CAVITY	(55)	(54)	(52)
NECROSIS, FAT	7 (13%)		
*MESENTERY	(55)	(54)	(52)
CYST, NOS	1 (2%)		
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(55)	(54)	(52)
PERIARTERITIS	1 (2%)		
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	1	1	2
ANIMAL MISSING/NO NECROPSY		1	1
AUTO/NECROPSY/HISTO PERF		2	1
AUTOLYSIS/NO NECROPSY			2

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

Review of the Bioassay of 2,3,5,6-Tetrachloro-4-Nitroanisole*
for Carcinogenicity
by the Data Evaluation/Risk Assessment Subgroup
of the Clearinghouse on Environmental Carcinogens

June 29, 1978

The Clearinghouse on Environmental Carcinogens was established in May, 1976, in compliance with DHEW Committee Regulations and the Provisions of the Federal Advisory Committee Act. The purpose of the Clearinghouse is to advise the Director of the National Cancer Institute (NCI) on its bioassay program to identify and to evaluate chemical carcinogens in the environment to which humans may be exposed. The members of the Clearinghouse have been drawn from academia, industry, organized labor, public interest groups, State health officials, and quasi-public health and research organizations. Members have been selected on the basis of their experience in carcinogenesis or related fields and, collectively, provide expertise in chemistry, biochemistry, biostatistics, toxicology, pathology, and epidemiology. Representatives of various Governmental agencies participate as ad hoc members. The Data Evaluation/Risk Assessment Subgroup of the Clearinghouse is charged with the responsibility of providing a peer review of reports prepared on NCI-sponsored bioassays of chemicals studied for carcinogenicity. It is in this context that the below critique is given on the bioassay of 2,3,5,6-Tetrachloro-4-Nitroanisole for carcinogenicity.

The reviewer noted an increased (though not statistically significant) incidence of hepatic neoplasms among treated rats and lymphomas and leukemias among treated mice. Although the results indicated that the animals were administered maximum tolerated doses of the compound, the reviewer expressed surprise that the levels were so low given the nature of the substance. He suggested that the low dose levels imposed by the toxicity may have limited the expression of a higher tumor rate at those sites at which an increased incidence was observed. Despite the apparent adequacy of the study, the reviewer felt that some additional testing was appropriate. In this regard, he suggested that short-term in vitro assays might provide useful information. The reviewer concluded that the bioassay

did not clearly show 2,3,5,6-Tetrachloro-4-Nitroanisole to be positive or negative under the conditions of test. His conclusion was accepted without objection.

Clearinghouse Members present:

Arnold L. Brown (Chairman), Mayo Clinic
Paul Nettesheim, National Institute of Environmental Health Sciences
Verne Ray, Pfizer Medical Research Laboratory
Verald K. Rowe, Dow Chemical U.S.A.
Michael B. Shimkin, University of California at San Diego
Louise Strong, University of Texas Health Sciences Center

---

* Subsequent to this review, changes may have been made in the bioassay report either as a result of the review or other reasons. Thus, certain comments and criticisms reflected in the review may no longer be appropriate.

☆U.S. GOVERNMENT PRINTING OFFICE: 1978-260-899/3182

DHEW Publication No. (NIH) 78-1369